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CONTENTS

Diffuse Interstitial Fibrosis of the Lungs (Hamman-Rich Syndrome): A Review with a Report of Three Additional Cases. I. W. B. Grant, B. R. Hillis, and J. Davidson 485

Simple Tests of Respiratory Function Using a Direct-Writing Ear Oximeter. C. R. Woolf, R. W. Gunton, and W. Paul 511

Beryllium Granulomatosis of the Lungs. A Four-Year Follow-Up of Two Patients. Arthur Schwartz, Sidney D. Harvey, and Daniel J. Stoke 533

Antituberculous Immunity Induced in Mice by Virulent Primary Infection. Its Inhibition by Chemoprophylaxis. René J. Dubos and Werner B. Schaeffer 541

Phagocytosis of *M. Tuberculosis* and *M. Smegmatis* Stained with Indicator Dyes. Marian G. Sprick 552

The Influence of Tetracycline Compounds on the Development of Antibodies in Psittacosis. K. F. Meyer and B. Eddie 566

Resistance of Tubercle Bacilli to Pyrazinamide *in Vivo*. Ole Wasz-Höckert, Robert M. McCune, Jr., Seung Hoon Lee, Walsh McDermott, and Ralph Tompsett 572

Complications Associated with Polyvinyl-Formal Sponge Prosthesis and Plombage in the Surgical Management of Pulmonary Diseases. Ezra V. Bridge, George S. Allen, F. Henry Ellis, Jr., and O. Theron Clagett 581

The Los Angeles County Jail Chest Roentgenographic Screening Program. Harvey I. Meyers, George Jacobson, and Frank W. Orchell 590

Retention and Differentiation of Carbolfuchsin-Stained Mycobacteria in Diagnostic Films. John H. Hanks 597

Retention and Differentiation of Mycobacteria in Tissue Sections. John H. Hanks 608

Cultivation of *M. Tuberculosis* from Urine and Gastric Lavage by the Pancreatin-Quaternary Ammonium Compound Method. Rolf Saxholm 616

(*Contents—continued on page 16 of advertisements*)

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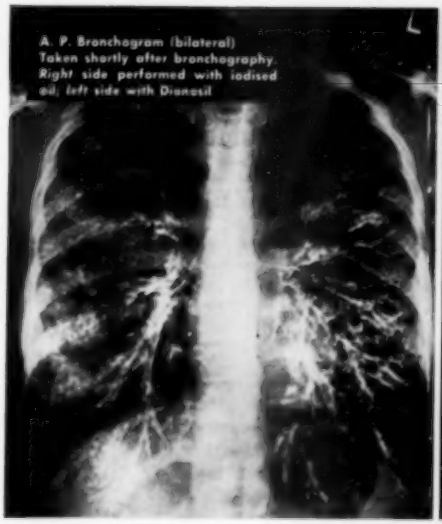

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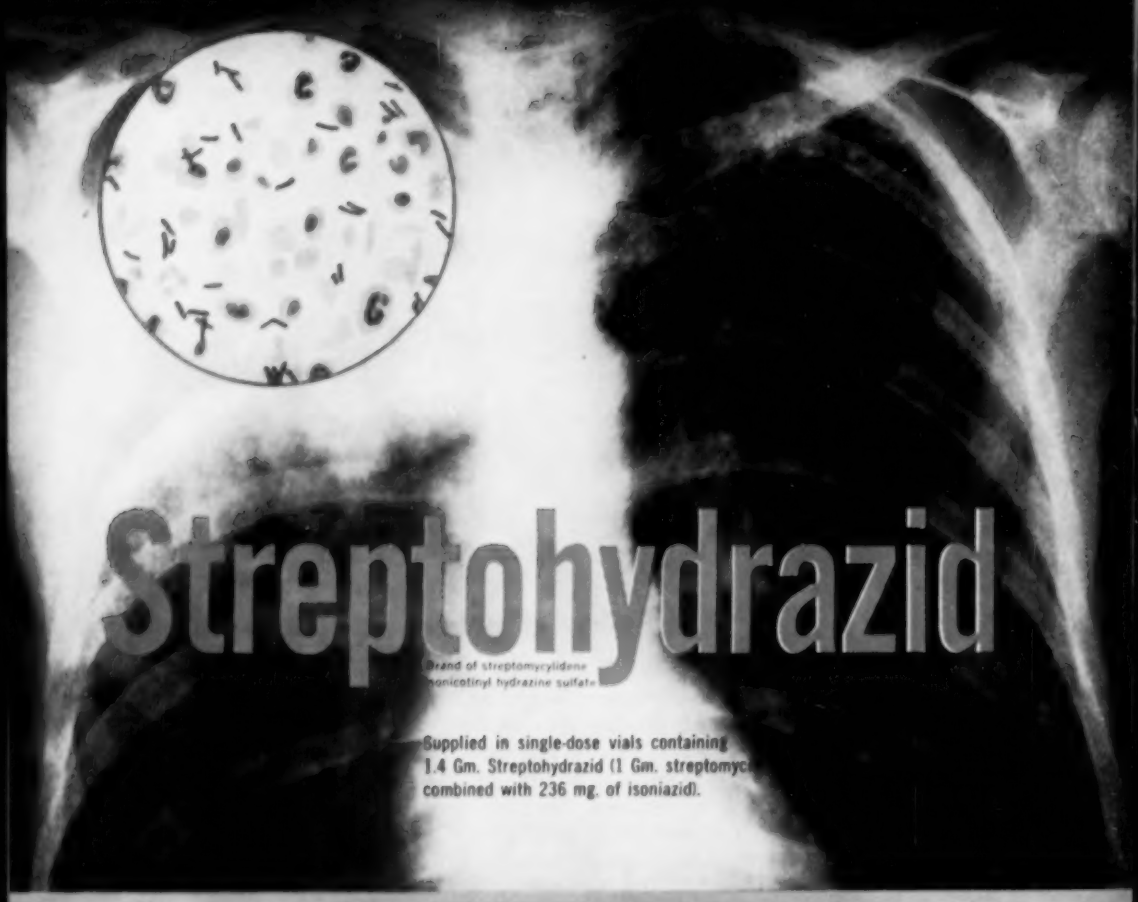

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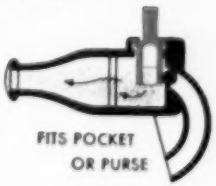

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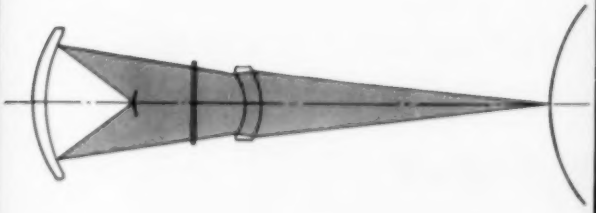

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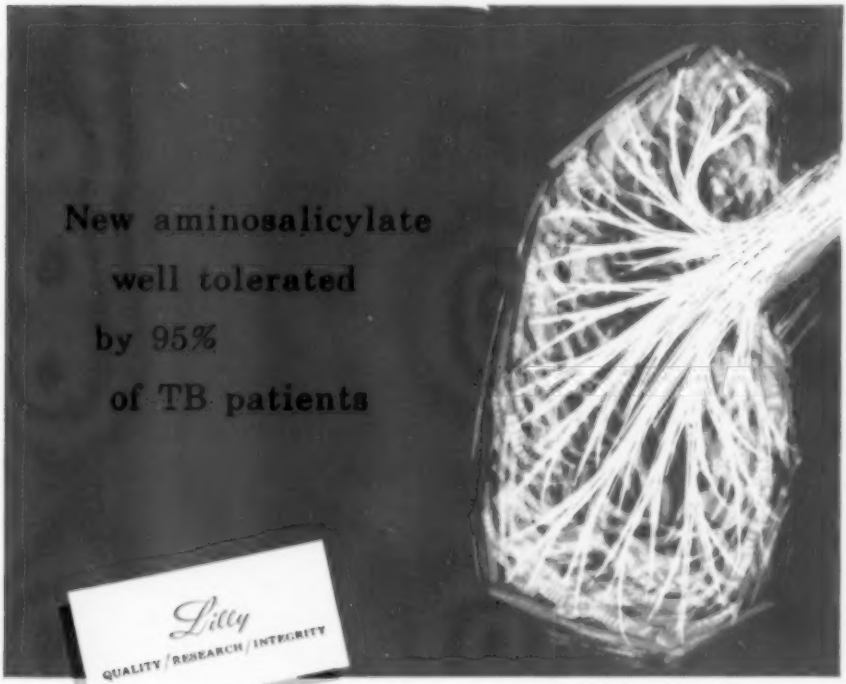

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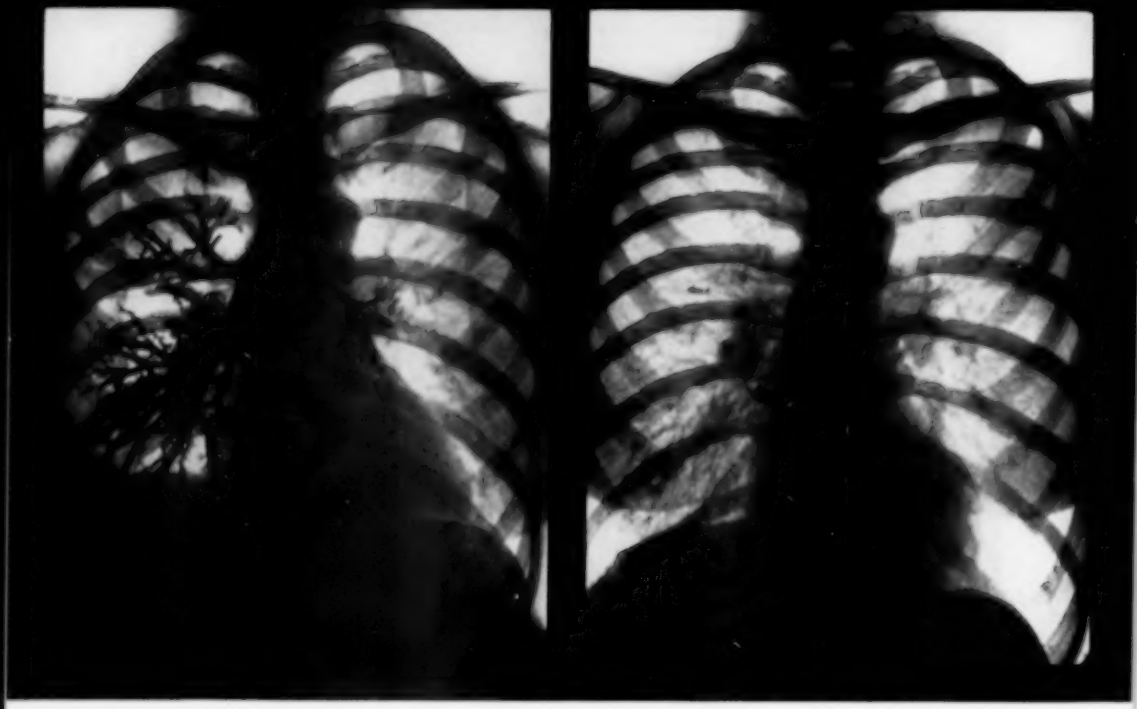

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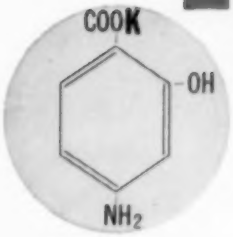

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 74 *October, 1956* NUMBER 4

DIFFUSE INTERSTITIAL FIBROSIS OF THE LUNGS (HAMMAN-RICH SYNDROME)[1]

A Review with a Report of Three Additional Cases

I. W. B. GRANT, B. R. HILLIS, AND J. DAVIDSON

(Received for publication June 22, 1956)

INTRODUCTION

In 1944 Hamman and Rich (1) reported four cases of diffuse interstitial fibrosis of the lungs conforming to a distinctive histologic pattern but not attributable to any known cause. Since then thirty-two similar cases have been recorded, chiefly by American writers. Most of the cases presented insoluble diagnostic difficulties and the nature of the condition was seldom suspected before autopsy. Nevertheless, in most instances, it was possible in retrospect to recognize, among all the diverse symptoms and signs which these patients presented, the cardinal feature of unremittingly progressive dyspnea.

Roentgenographically, diffuse bilateral pulmonary changes were invariably present although the nature of these varied considerably from case to case. The most common abnormalities reported were either ill-defined patchy shadowing or irregularly distributed strandlike opacities. In several instances both types of abnormality were found.

The most inconsistent feature in the clinical description of the various cases was the length of the illness. In the four cases of Hamman and Rich (1) the interval between the onset of symptoms and death varied from one to six months. In most of the cases subsequently reported, however, the disease pursued a more chronic course, extending on an average to almost two and a half years.

Apart from one in which the diagnosis was made by means of lung biopsy (2), in every instance so far recorded, chronic as well as acute, the patient has died from the disease or its complications. The 3 cases to be described in this paper also had a fatal outcome.

The pathologic findings on which the diagnosis of diffuse interstitial fibrosis of the lungs is based are essentially histologic, the two characteristic changes being (*a*) thickening of the alveolar walls by proliferating fibrous tissue and (*b*) an increase in the size and number of the alveolar epithelial cells with, es-

[1] From the Departments of Tuberculosis and Pathology, University of Edinburgh, Edinburgh, Scotland.

pecially in acute cases, a partial or complete hyaline membrane lining the alveoli. The diagnosis is, of course, invalidated if the changes are not distributed more or less diffusely throughout both lungs or if the patient has any other disease, such as pneumoconiosis, capable of producing interstitial fibrosis.

During the past three years, in a small unit for the treatment of respiratory diseases, 3 cases of diffuse interstitial fibrosis of the lungs have been encountered which fulfilled the histologic criteria of Hamman and Rich. In recording these cases, the opportunity is taken of examining in detail the clinical course of the illness, the roentgenographic changes, the pathologic features, and the current views on etiology and treatment.

CASE REPORTS

Case 1. E.M., female, aged sixty-nine years.

Eighteen months before admission the patient had an acute respiratory illness, starting with cough productive of a little sputum and later associated with breathlessness on exertion and dependent edema. She was treated for congestive cardiac failure with rest, digitalis, and mercurial diuretics for about six weeks. This treatment controlled the edema but the cough persisted; the breathlessness, after an initial slight improvement, became slowly but progressively worse until, when she was admitted to the hospital, the dyspnea was present even at rest.

On admission to the hospital, the patient was well nourished and afebrile but was orthopneic, moderately cyanosed, and edematous. There was marked clubbing of the fingers and toes. Respiration was rapid and shallow. Numerous crepitations were audible over both lungs, particularly at the bases, with a few scattered rhonchi. Examination of the cardiovascular system showed distension of both external jugular veins, multiple extra-

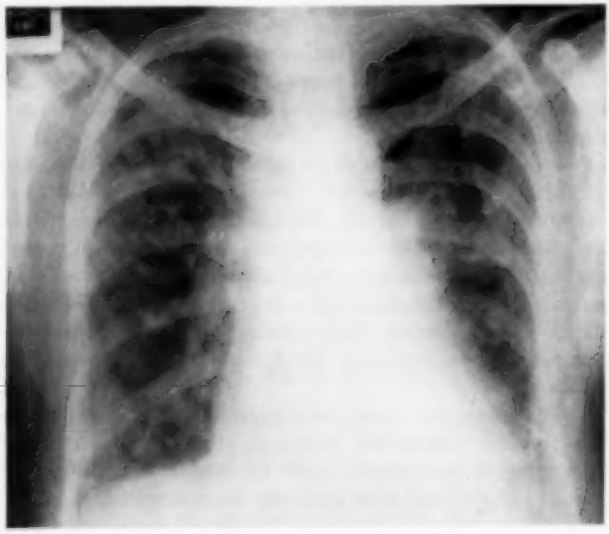

FIG. 1. *Case 1.* Postero-anterior roentgenogram showing streaky and patchy opacities throughout both lung fields, most marked in right upper and both lower zones.

systoles, a loud mitral first sound, an added sound in mid-diastole, a systolic murmur over the whole precordium (maximal at the apex), and an accentuated pulmonic second sound. The blood pressure was 135 systolic, 70 diastolic in mm. of mercury. Examination of the other systems was negative.

The chest roentgenogram (figure 1) showed streaky and patchy, partly confluent, opacities throughout both lung fields, most marked in the right upper and both lower zones. Both hilar shadows were abnormally prominent. There was slight general enlargement of the heart shadow and some aortic calcification. The diaphragm on both sides was partially obscured by the pulmonary opacities. On tomography the opacities were seen to be present throughout the whole depth of both lungs, being ill defined but apparently dissociated from the normal vascular shadows.

The sputum was mucoid and contained no tubercle bacilli on microscopy or culture or any other customarily pathogenic organisms. Gastric washings were also negative for tubercle bacilli. The Mantoux reaction was negative to 0.1 ml. of 1:100 (1 mg.) of Old Tuberculin. The erythrocyte sedimentation rate was 46 mm. in the first hour (Westergren). Electrocardiograms showed right ventricular hypertrophy and possibly also an old anterior myocardial infarct.

The patient was treated with rest, a low salt diet, digitalis, and mercurial diuretics, with some improvement in the edema but no change in the breathlessness or in the physical signs. Her temperature remained normal and her sputum mucoid. Penicillin and later streptomycin, PAS, and isoniazid were given empirically without effect on her symptoms or roentgenographic course. Approximately ten weeks after admission the dyspnea suddenly increased in severity. The patient became deeply cyanosed and died a few hours later.

At autopsy the abnormal findings were confined to the thoracic organs. The trachea and bronchi were normal. No significant pleural effusion and no pleural adhesions were noted, but there was patchy fibrous thickening on the pleural surfaces of both lungs. The lungs were of normal size and shape but had a firm, tough consistence. On section they were almost completely consolidated, the cut surface varying in color from dark red to almost

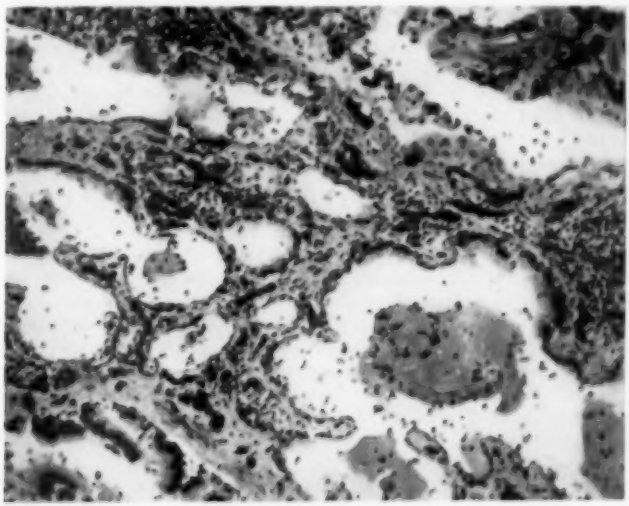

FIG. 2. *Case 1.* Lung section (× 110) showing air spaces of varying size lined by columnar epithelium. Some may be bronchioles but the majority are probably alveoli.

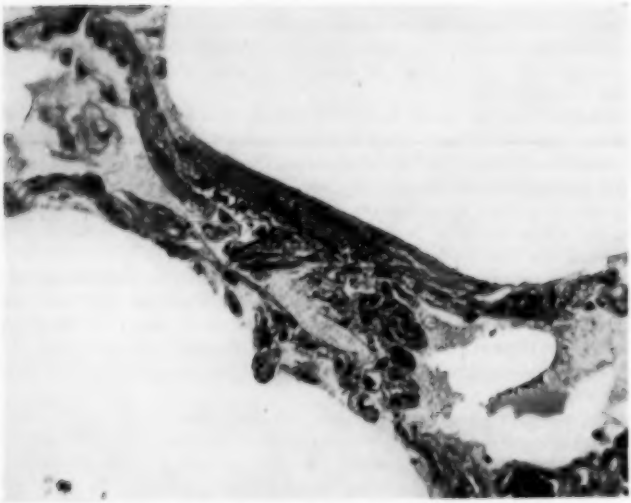

FIG. 3. *Case 1.* Lung section (× 300) showing alveolus partially lined by a hyaline membrane.

pure white. The right upper lobe consisted entirely of white tumor-like tissue with occasional small intervening areas of edema. Elsewhere the pale areas were rather less extensive, but small white nodules could be distinguished beneath the pleural surfaces of all lobes.

Microscopic examination showed widespread fibrosis affecting most of the parenchyma of both lungs but varying in degree from place to place and leaving some areas relatively uninvolved. The fibrous tissue was dense and mainly collagenous, with inflammatory cells distributed throughout it in varying numbers, mainly lymphocytes but with some plasma cells and histiocytes and occasional polymorphonuclears. Within the denser areas of fibrosis were air spaces of different sizes, some very large, the majority lined by cuboidal or even columnar epithelium (figure 2). Some appeared to be bronchioles but many were alveoli. Early squamous metaplasia had occurred in some areas. The lung parenchyma between the aggregations of fibrosis was congested and the alveolar walls were thickened, in some instances by fibrosis but mainly by histiocytic proliferation. Many of the alveoli showed an irregular hyaline lining (figure 3).

Histiocytes were present in clumps in many of the surviving alveoli, some containing hemosiderin. The large- and medium-sized bronchi were compressed and distorted by the surrounding fibrous tissue. Some of them showed small projections of mucosa into the lumen while others exhibited hyperplasia and squamous metaplasia of the epithelium. In none of the sections examined was there any evidence of tuberculosis, sarcoidosis, or pulmonary infarction. The larger pulmonary arteries displayed a well-marked atheroma. Among the areas of fibrosis marked thickening of the walls of the smaller arteries and arterioles was observed. This was due to very extensive fibrous thickening of the subintimal connective tissue. Proliferation of elastic tissue was noted occasionally, particularly near the internal elastic lamina. The lumen of many of the vessels was considerably reduced in size. In some cases there had been complete obliteration of the lumen which had been followed by recanalization.

On microscopic examination, the mediastinal and hilar lymph nodes showed small numbers of tuberculous follicles, some almost healed; others, recent, cellular, and active. Caseation was present in the more recent cellular follicles. In tissue stained by the Ziehl-Neelsen method, no acid-fast bacilli were found.

The heart was moderately enlarged and globular in shape. The enlargement was entirely

right-sided, both right ventricle and right atrium being considerably dilated. The right ventricle was also hypertrophied, its wall being about one and one-half times the normal thickness. There was only slight hypertrophy of the right atrial wall. A small area of ischemic fibrosis was present on the anterior wall of the left ventricle near the apex. The aortic valve showed mild atheromatous thickening at the bases of the cusps, but the other valves were normal. The anterior descending branch of the left coronary artery was markedly atheromatous and showed gross narrowing and calcification.

Death was considered to be due to diffuse interstitial fibrosis of the lungs complicated by cor pulmonale.

Case 2. M.B., female, aged seventy-two years.

Nine months before admission this patient had an acute febrile illness, accompanied by cough productive of mucoid sputum which was blood-stained on one occasion. She was treated with penicillin with only limited improvement and thereafter developed progressive breathlessness on exertion, with lassitude and anorexia. One month before admission she again became febrile and there was a marked increase in her cough and sputum which, however, remained mucoid in character.

On admission to the hospital she was thin and in poor general condition, but she was afebrile. She became breathless on the slightest exertion but was not orthopneic. There was no cyanosis or edema. Marked clubbing of the fingers was present. The respiratory rate was increased to 28 per minute. Scattered crepitations and a few rhonchi were present over both lower lobes. A regular tachycardia of 100 per minute was noted, but no signs of cardiac failure or any significant abnormality on auscultation were present. Blood pressure was 100 systolic, 70 diastolic in mm. of mercury. Examination of the other systems was negative.

The chest roentgenogram (figure 4) showed patchy and streaky opacities throughout both lung fields, partly confluent in the right upper zone and at both bases, superimposed on a background of coarse reticular shadowing. Both hilar shadows were abnormally prominent. The heart shadow was normal. The diaphragmatic outline on both sides was partially obscured by the pulmonary opacities.

FIG. 4. *Case 2.* Postero-anterior roentgenogram showing streaky and patchy opacities throughout both lung fields, partly confluent in right upper zone and at both bases, superimposed on a background of coarse reticular shadowing.

The sputum was mucoid and contained no tubercle bacilli on concentration or culture or any other pathogenic organisms. The Mantoux reaction was negative to 0.1 ml. of 1:100 (1 mg.) of Old Tuberculin. The erythrocyte sedimentation rate was 50 mm. in the first hour (Westergren). Electrocardiograms showed a partially stabilized pattern of an anterior myocardial infarct.

The patient's condition remained unchanged until a week after admission when she became febrile to 100° to 101°F. The pyrexia failed to respond to sulfadimidine but subsided gradually after three days of treatment with penicillin. No cause for the pyrexia was discovered, the sputum remained mucoid, and the degree of breathlessness was unchanged. Five weeks after admission a pleural rub appeared at the right lung base. The following day the patient suddenly complained of a severe anterior chest pain and a few minutes later collapsed and died.

At autopsy the abnormal findings were confined to the thoracic organs. The trachea and bronchi were normal. No significant pleural effusion and no pleural adhesions were observed, but a large patch of pleural thickening was present over the right upper lobe with some recent fibrinous pleurisy. Both lungs were rather small and of rubbery consistence. On section they showed a diffuse fibrosis, more marked towards the apices in which a few firmer nodules containing much anthracotic pigmentation were noted. The bronchi and bronchioles were slightly dilated, particularly in the upper lobe, but contained no pus. There was no evidence of pneumonic consolidation, infarction, tuberculosis, or sarcoidosis.

The pulmonary artery and its major branches on both sides showed patchy atheroma, and an organizing adherent thrombus was noted, estimated to be of some weeks' standing, partially occluding the right pulmonary artery and some of its main branches.

Microscopic examination of tissue from both lungs showed an extreme degree of fibrosis which was most marked at the periphery, where it almost completely obliterated the pulmonary architecture. The alveoli which remained showed fibrous thickening of their walls in varying degrees. The lining cells in many of these alveoli were prominent and in some they were cuboidal in shape. In several areas, groups of alveoli were lined by columnar epithelium, making them difficult to distinguish from bronchi. Collections of such alveoli gave a picture resembling adenomatous growth. None of the alveoli in this case showed any hyaline lining. Histiocytes and a few lymphocytes and plasma cells were present in many of the alveoli, but no polymorphonuclear leukocytes. In some areas where the alveolar walls did not show such advanced fibrosis, young fibrous tissue could be seen passing from within one alveolus to another, an appearance resembling that seen in organizing pneumonia, but this was not general. Aggregations of lymphoid tissue were present here and there in the thickened alveolar walls as well as numbers of plasma cells and a few eosinophils. Certain of the small arteries and arterioles showed subintimal fibrous thickening with an increase of elastic fibers. This was, however, not marked and occurred only in those areas where the pulmonary fibrosis was most advanced.

The mediastinal and hilar lymph nodes were slightly enlarged but showed no specific abnormality. The heart was not enlarged but the right ventricular wall showed moderate hypertrophy. There was an extensive healed infarct in the left ventricular wall but no recent infarction. The coronary arteries showed marked atheroma and calcification.

Death was considered to be due to diffuse interstitial fibrosis of the lungs, the fatal outcome being precipitated by pulmonary arterial thrombosis.

Case 3. J.F., female, aged thirteen years.

The patient, apart from measles when eight months old, was well until three years before admission when she developed a cough, which later became paroxysmal and at times emetic. Only a small amount of mucoid sputum was produced. She became progressively more breathless on exertion and tired, lost her appetite, and lost weight. She was admitted to the hospital and her symptoms improved except the breathlessness which steadily became worse. Two years before admission her condition again deteriorated but improved partially with a further period in the hospital. This improvement was, however, not maintained and she became emaciated and cyanosed as well as breathless on the least exertion.

FIG. 5. *Case 3.* Postero anterior roentgenogram showing streaky and patchy opacities throughout both lung fields, more marked on the right side, with gross distortion and rotation of mediastinal shadow.

On admission she was thin and markedly cyanosed, but not breathless at rest. There were no signs of cardiac failure. The fingers were clubbed. The chest was markedly flattened anteroposteriorly and expansion was negligible. The breath sounds were reduced in intensity with no accompaniments. There was a persistent tachycardia of 100 to 130 per minute, a right ventricular heave, and a markedly accentuated pulmonic second sound with a visible impulse in that area. The blood pressure was 120 systolic, 90 diastolic in mm. of mercury. Examination of the other systems was negative.

The chest roentgenograms taken from the start of the illness were available. These showed scattered patchy and streaky pulmonary opacities with pleural thickening, at first mainly on the left side but a year later becoming almost equally extensive on the right. These changes became gradually more marked, with progressive rotation of the mediastinum to the left (figure 5). The hilar shadows were normal and there was no cardiac enlargement. The right hemidiaphragm was obscured by the pulmonary opacities; the left was normal. Bronchoscopy a year before admission was reported to have been negative. Bronchograms obtained at the same time showed some general shrinkage of the left bronchial tree but no bronchiectasis. The sputum was mucoid and did not contain tubercle bacilli on microscopy or culture, but a scanty growth of hemolytic streptococci was obtained on one occasion. The Mantoux reaction was negative to 0.1 ml. of 1:10 (10 mg.) of Old Tuberculin. The erythrocyte sedimentation rate was 14 mm. in the first hour (Westergren). The electrocardiogram was normal, except for sinus tachycardia. Aspiration liver biopsy showed no evidence of sarcoidosis.

While in the hospital the patient's general condition improved slightly and she gained weight, but her dyspnea on exertion did not improve and she could not walk more than 50 yards on the level without distress. She was consistently afebrile and produced no sputum after the second day in the hospital. At the end of five weeks she was temporarily discharged for the Christmas holidays. It was intended later to treat her with cortisone, but she died suddenly at her home four hours after the onset of acute dyspnea at rest.

At autopsy the abnormal findings were confined to the thoracic organs. The mucosa of the larynx, trachea, and bronchi was markedly congested and the lumen of the trachea was filled with blood-stained mucopurulent exudate. No significant pleural effusion and no pleural adhesions were noted. Both lungs showed opaque fibrous thickening of the pleural surface which in some areas presented a smooth, glistening white appearance (figure 6). The surface of the lungs was lobulated, resembling that of an atrophic cirrhotic liver. There was a large area of depressed scarring in the left upper lobe, including the apex, and there were numerous small pitted scars elsewhere on the lung surfaces. Both lungs were of firm consistence and cut with difficulty. On section they had an almost uniform, pale, fibrous appearance.

Microscopic examination of both lungs showed extensive fibrosis of the interstitial type (figures 7*A* and *B*), basically diffuse but varying in degree in different areas from slight thickening of the alveolar walls to total replacement of the alveolar tissue by fibrosis. In most areas where alveolar spaces remained they were partially obliterated by their thickened walls. In some of the alveoli the lining cells were swollen and cuboidal or even columnar in shape; in others there was a definite epithelial proliferation. Some alveoli showed a thick hyaline membrane partially lining their walls. Edema was present in some areas, including the alveolar septa, and there was marked generalized congestion. Numerous collections of small lymphocytes were distributed among the denser areas of fibrosis, and occasional eosinophils were seen. There was no inflammatory exudate in the alveoli, and no evidence of tuberculosis or sarcoidosis was present. No marked fibrous thickening of the vessel walls was observed. Certain of the vessels showed some increase in elastic tissue in the media, extending into the adventitia. Tissue was taken from the lungs for attempts at isolation of a virus, but the results were negative.

FIG. 6. *Case 3.* Lung showing gross pleural thickening, with a pitted and lobulated surface due to underlying pulmonary fibrosis.

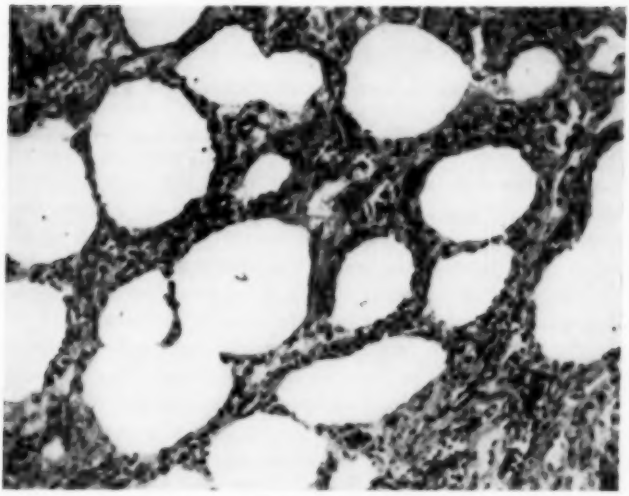

FIG. 7A. *Case 3.* Lung section (× 120) showing diffuse fibrosis of the alveolar walls.

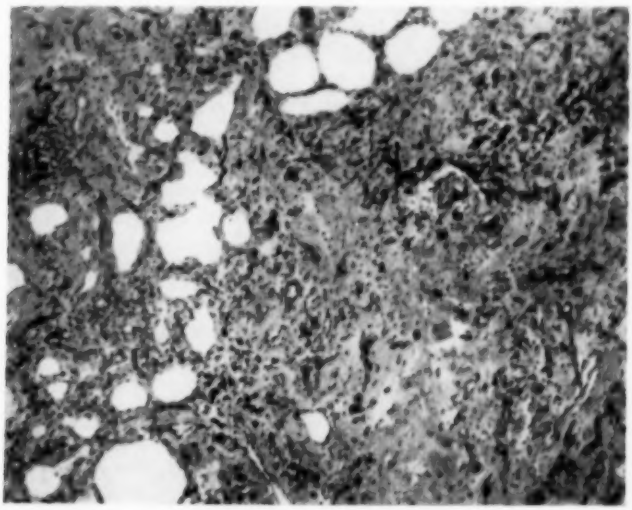

FIG. 7B. *Case 3.* Lung section (× 80) showing areas of massive fibrosis. The walls of the few surviving alveoli are grossly thickened by fibrous tissue.

The mediastinal and hilar lymph nodes were slightly enlarged and contained on section numerous pale areas of pin-point size shown on microscopic examination to be dilated lymph sinusoids containing numerous polymorphonuclear leukocytes. There was no evidence of tuberculosis or sarcoidosis.

The heart was slightly enlarged and the right ventricle was considerably dilated and

hypertrophied. Death was considered to be due to acute tracheobronchitis supervening on diffuse interstitial fibrosis of the lungs.

REVIEW OF CLINICAL AND ROENTGENOGRAPHIC FEATURES

Present Series

From the clinical point of view the patients all presented with progressive dyspnea although none of them showed the rapid deterioration described by Hamman and Rich (1) and only 2 were cyanosed. In all 3 cases the onset of breathlessness was either preceded or accompanied by cough productive of mucoid sputum. In 2 patients the cough was severe and paroxysmal. Only one was febrile while under observation, and in none was there any frank evidence of pyogenic respiratory infection, except terminally in *Case 3*.

All 3 patients had definite finger clubbing, although none of the conditions commonly associated with this abnormality was found. The physical signs in the chest in all cases were unhelpful although 2 patients had persistent bilateral crepitations, predominantly basal. Roentgenographically the appearances were similar in all 3 cases, consisting of strandlike and patchy opacities distributed irregularly throughout both lung fields. There was no "honeycombing." In only one case was there any clear roentgenographic evidence of pleural thickening although in all cases it was found post mortem. In 2 cases the hilar shadows were abnormally prominent. In the third there was no enlargement but the mediastinal shadow was grossly distorted and rotated. In all 3 patients the Mantoux reaction was negative to 1 mg. of Old Tuberculin, and in one patient the reaction was also negative to 10 mg. There was nothing further to support a diagnosis of sarcoidosis in any of the cases. There was no enlargement of the superficial lymph nodes, liver, or spleen. Roentgenographic examination of the hands and feet was negative. The total plasma protein in *Case 2* was raised to 9.25 gm. per 100 ml. but no frank disturbance in the albumin-globulin ratio occurred in any of the patients. The serum alkaline phosphatase was slightly elevated in 2 patients, one with congestive cardiac failure. The peripheral blood was normal except for considerable elevation of the erythrocyte sedimentation rate in 2 cases. Right ventricular hypertrophy was apparent clinically in one patient, and electrocardiographically in another, but was found at autopsy in all 3, although only one patient had congestive cardiac failure (*Case 1*).

The time from the first symptoms until death in the 3 cases was eighteen months, nine months, and three years. The immediate cause of death in all 3 patients was respiratory insufficiency which, in *Case 2*, was aggravated by pulmonary arterial thrombosis and, in *Case 3*, by acute tracheobronchitis.

Previously Recorded Cases (Table 1)

Since the first description of the disease by Hamman and Rich (1), thirty-two cases have been reported which seem to be examples of the same condition, making a total of thirty-six in all. Cases have been excluded from the present review if the pathologic diagnosis seemed doubtful or if the descriptions of the

autopsies were incomplete. Only three cases have so far been reported by British authors (3–5). The remainder have occurred mainly in the United States (1, 2, 6–24).

Age, sex, and race incidence: In the thirty-six documented cases, the age at the onset of dyspnea ranged from 25 to 72 years, with an average of 44.5 years. The cases were divided almost equally between the sexes. The disease was reported in both whites and nonwhites.

Previous respiratory illnesses: In only eleven of thirty-six cases was there any history of previous respiratory disease. The diagnosis recorded in these cases included conditions such as pneumonia, pleurisy, tuberculosis, asthma, and upper respiratory tract infections, none of them apparently being related to the later illness.

Clinical features: The onset of symptoms was acute in eight cases, subacute in twelve, and insidious in sixteen. In some of the "acute" cases it was difficult to be certain from the descriptions given whether the acute illness represented the start of the disease or merely the exacerbation of previously mild symptoms. When the symptoms began fairly abruptly, the pattern at the onset often resembled that of a moderately severe respiratory infection. No response to the usual measures of treatment would occur, however, and unremittingly progressive dyspnea soon became the dominant feature of the illness. When the onset was more gradual, increasing breathlessness on exertion was generally the symptom which caused the patient to seek medical advice.

Whether the onset was sudden or gradual, cough was almost invariably present. In some cases it started before the dyspnea and in a few it came later, but in the majority the two symptoms appeared at approximately the same time. Some sputum was often produced but its amount and character were variable. In most cases, at some stage the sputum was purulent but it is not clear whether this was an inherent feature of the illness or due to an added pyogenic infection. Hemoptysis occurred in almost one-quarter of the cases. The hemoptysis was usually "frank" but seldom of large amount. Wheezing was noted in only a few instances, and one of these patients had been suffering from chronic bronchitis for many years. Other symptoms frequently complained of were tiredness, weakness, and loss of weight.

In most cases the patients' general condition on admission to the hospital was still fairly good and few were grossly underweight. The degree of dyspnea varied considerably but was usually incapacitating, although only a few patients had orthopnea at that stage. Cyanosis was noted in thirty-three cases and frank congestive cardiac failure in sixteen. Finger clubbing was noted in fourteen of the sixteen cases in which the presence or absence of this feature was specifically recorded. Pyrexia developed at some stage in twenty patients but only for short periods. The physical signs in the chest conformed to no single pattern. Diminished respiratory movement, harsh breath sounds, and crepitations or rhonchi were those most frequently described, and they were almost invariably bilateral. The second pulmonic sound was accentuated in twelve instances and an added

TABLE 1

DIFFUSE INTERSTITIAL FIBROSIS OF THE LUNGS; ABSTRACT OF PROVED CASES IN THE LITERATURE AND IN THE PRESENT SERIES

Writer	Year Reported	Age at Onset of Dyspnea	Sex	Mode of Presentation	Cyanosis	Clubbing	Sputum	Hemoptysis	Cardiac Failure	Right Ventricular Hypertrophy at Post-Mortem Examination	Febrile Episodes	Mode of Death	Period of Survival from Onset of Dyspnea
Hamman and Rich (1)	1944	47	M	Acute	Present	Not recorded	Some	Present	Present	Present	Present	Cardiac failure predominant	4 months
		25	F	Acute	Present	Not recorded	Some	Present	Present	Present	Present	Respiratory and cardiac	1½ months
		37	F	Acute	Present	Not recorded	Up to 30 ml per day	Present (also pulmonary tuberculosis)	Absent	Present	Present	Respiratory	1 month
		68	F	Subacute	Present	Not recorded	Some	Absent	Absent	Absent	Present	Respiratory	6 months
Eder, Van Zandt Hawn, and Thorn (6)	1945	47	M	Subacute	Present	Present	None	Absent	Absent	Present	Present (terminal)	Respiratory	4½ months
Potter and Gerber (7)	1948	33	M	Insidious	Present	Not recorded	Not recorded	Absent	Absent	Present	Present (terminal)	Respiratory	About 6 months

Beams and Harmos (8)	1949	32	M	Sub-acute	Present	Present	Some	Present	Present	Present	Present (terminal)	Respiratory	11½ months
Golden and Tullis (9)	1949	41	M	Insidious	Present	Present	150–200 ml. per day	Absent	Present	Present	Absent	Respiratory and cardiac	6–7 years
		42	F	Sub-acute	Not recorded	Not recorded	Not recorded	Not recorded	Present	Present	Present (terminal)	Cardiac failure predominant	5½ months
Peabody, Peabody, Hayes, and Hayes (10) (twins)	1950	43	F	Insidious	Present	Not recorded	Some	Present	Absent	Absent	Present (terminal)	Respiratory	3½ years
		42	F	Insidious	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	? Respiratory	5 years
Katz and Auerbach (11)	1951	50	M	Insidious	Present	Present	Some	Absent	Present	Absent	Present	Cardiac failure predominant	3 years
Heppleston (3)	1951	25	F	Acute	Present	Present	Some	Present	Present	Present	Absent	Respiratory	14 months
Peabody, Moersch, and Edwards (12)	1951	47	F	Insidious	Present	Present	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	?Respiratory	2 years

TABLE 1—*Continued*

Writer	Year Reported	Age at Onset of Dyspnea	Sex	Mode of Presentation	Cyanosis	Clubbing	Sputum	Hemoptysis	Cardiac Failure	Right Ventricular Hypertrophy at Post-Mortem Examination	Febrile Episodes	Mode of Death	Period of Survival from Onset of Dyspnea
Rubin, Kahn, and Pecker (13)	1952	44	F	Subacute	Present	Present	Some	Absent	Present	Absent	Present	Respiratory	2½ years
Callahan, Sutherland, Fulton, and Kline (14)	1952	55	M	Insidious	Present	Absent	Some	Present	Present	Not recorded	Present on cortisone	Respiratory	2 months
Cox and Kohl (15)	1952	59	F	Acute	Present	Absent	Some	Absent	Not recorded	Absent	Present	Respiratory	3 years
Ferrar *et al.* (16)	1952	63	F	Subacute	Present	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	Not recorded	Respiratory	4 months
		41	M	Insidious	Present	Not recorded	Some	Not recorded	Not recorded	Not recorded	Not recorded	Respiratory	Several years
Golden and Bronk (17)	1953	50	M	Acute	Present (probably at least terminally)	Not recorded	Some	Absent	Not recorded	Present	Present (terminal)	Respiratory	"Long time"; acute for 9 days
		50	M	Subacute	Present	Present	Not recorded	Absent	Present	Not recorded	Absent	Respiratory and cardiac	? Several months; acute for about one week

Golden and Bronk (17) (con't)	1953	63	M	Insidious	Present	Not recorded	Some	Present	Not recorded	Not recorded	Present (terminal)	Respiratory	? About 4 months (probably much longer)
Schechter (18)	1953	31	M	Acute	Present	Not recorded	Some	Absent	Present	Absent	Present	Respiratory	6-7 months
		38	M	Insidious	Present	Not recorded	15-30 ml. per day	Absent	Present	Not recorded	Absent	Respiratory	5 years
Peabody, Buechner, and Anderson (19)	1953	31	M	Subacute	Present	Not recorded	Some	Absent	Absent	Absent	Present	Respiratory	5½ months
		72	F	Insidious	Present (terminal)	Not recorded	Not recorded	Absent	Absent	Not recorded	Present (terminal)	Respiratory	Dyspnea only in last week (other symptoms for 2 years)
		38	M	Insidious	Present	Present	Some	Absent	Absent	Present	Absent	Respiratory	9 years
Silverman and Talbot (20)	1953	36	M	Subacute	Present	Present	Absent	Absent	Present	Present	Absent	Cardiac failure predominant	20 months

TABLE 1—*Continued*

Writer	Year Reported	Age at Onset of Dyspnea	Sex	Mode of Presentation	Cyanosis	Clubbing	Sputum	Hemoptysis	Cardiac Failure	Right Ventricular Hypertrophy at Post-Mortem Examination	Febrile Episodes	Mode of Death	Period of Survival from Onset of Dyspnea
Kirshner, Breckenridge, Allbritten, and Theodos (21)	1954	57	M	Insidious	Present	Present	Not recorded	Absent	Present	Present	Absent	Cardiac failure predominant	3 years
Ogilvie and Hulse (4)	1954	35	M	Subacute	Present	Not recorded	Some (terminal)	Absent	Present	Not recorded	Absent	Respiratory and cardiac	?4–5 months
Pokorny and Hellwig (22)	1955	72	M	Acute	Present	Not recorded	Some	Absent	Present	Present	Absent	Respiratory and cardiac	5 weeks
Dias da Costa and Torres (23)	1955	27	F	Subacute	Present	Present	Some	Absent	Present	Present	Terminal	Respiratory	2 years
Sloper and Williams (5)	1955	36	M	Insidious	Present	Present	Not recorded	Absent	Terminal	Present	Present	Respiratory and cardiac	6 years
Wildberger and Barclay (24)	1955	51	M	Insidious	Present	Not recorded	Some	Absent	Absent	Absent	Absent	Respiratory	12 months
		37	M	Insidious	Present	Present	Some	Absent	Absent	Present	Absent	Respiratory	9 years

Pinney and William Harris (2)	1956	27	F	Sub-acute	Not recorded	Not recorded	Some	Not recorded	Not recorded	Still alive	Absent	Still alive	17 months (to date)
Grant, Hillis, and Davidson (present communication)	1956	69	F	Acute	Present	Present	Some	Absent	Present	Present	Absent	Respiratory and cardiac	19½ months
		72	F	Acute	Absent	Present	25 ml. per day	Present	Absent	Present	Present	Respiratory	9 months
		13	F	Insidious	Present	Present	Some	Absent	Absent	Present	Absent	Respiratory	3 years

heart sound was heard in five. While right ventricular hypertrophy was a common finding at autopsy, it was seldom apparent clinically. The blood pressure was usually normal.

Physical examination of the other systems was essentially normal. In a few cases some "soft" enlargement of the superficial lymph nodes was mentioned but in no case was splenic enlargement recorded. The liver was enlarged only in patients with congestive cardiac failure.

Special investigations: It was difficult to form a clear impression of the roentgenographic changes as their nature does not lend itself to satisfactory reproduction and the verbal descriptions were often confusing. The impression gained was that all patients had diffuse bilateral opacities; some of them strandlike, others patchy or nodular. Hilar or pleural abnormalities were seldom mentioned. Sputum in all cases was negative for tubercle bacilli and no other pathogens were isolated with any regularity. The tuberculin reaction was positive in 54 per cent of patients in whom it was recorded. A significant degree of anemia was never noted but a few patients exhibited mild polycythemia, especially during the later stages of the illness. The erythrocyte sedimentation rate was usually raised. Many biochemical estimations were carried out, including the plasma proteins and serum alkaline phosphatase. In none was any significant abnormality found. Detailed pulmonary function or blood gas studies were not undertaken except by Silverman and Talbot (20) in a single case. They found normal ventilation associated with a slight reduction of arterial oxygen saturation at rest. Electrocardiographic findings were recorded in nineteen of the thirty-six cases, but detailed accounts were given only in the more recent reports. In eight instances there was acceptable evidence of cor pulmonale; in another three there were changes suggestive of that condition; in the remainder the reports were either negative or uninformative. At autopsy definite right ventricular hypertrophy was found in seventeen cases, almost 50 per cent of the total.

Ultimate outcome: In every case so far reported with but one exception (2) the patient has died. This may not accurately reflect the true mortality, as at present the diagnosis is seldom established during life and, in consequence, it is almost exclusively the fatal cases which are recorded. It is possible that in some cases the disease pursues a benign course and the patient might die from another cause.

Respiratory insufficiency accounted for the majority of deaths. Death was often preceded by a sudden and marked increase in dyspnea, sometimes associated with an acute pyogenic respiratory infection or pulmonary heart failure which occurred independently or together. In other deaths, there was no clear cause. The time between the onset of symptoms and death varied considerably. In Hamman and Rich's original series (1) one patient lived for only a month, while in a case reported by Peabody, Buechner, and Anderson (19) the patient survived for nine years. As almost all writers on this subject have pointed out, Hamman and Rich appeared to limit the diagnosis of diffuse interstitial fibrosis of the lungs to patients whose illness pursued a rapidly fatal course, their longest

survivor living for only six months. Patients with the disease recorded in the subsequent literature have had much longer survival periods, the average being a little more than two years from the onset of dyspnea.

REVIEW OF PATHOLOGIC FEATURES

Present Series

At autopsy the 3 new cases reported in detail above showed similar pathologic features. Macroscopically the lungs were of normal size but had a firm, tough consistence. There were plaques of organized pleurisy on some parts of the lung surfaces; elsewhere there was much pitting and scarring from underlying pulmonary fibrosis. On section it was difficult to distinguish any normal lung substance, the bulk of it presenting the gross appearance of either fibrosis or consolidation. When the former predominated, it took the form of diffusely distributed strands or nodules. When the lung appeared to be consolidated, further scrutiny suggested that this was, in the main, due to the presence of more severe massive and confluent fibrosis than in other areas.

The principal microscopic abnormality in all 3 cases was that of interstitial fibrosis which, on the whole, was diffuse in character although varying in density from one area to another. Another conspicuous feature was an alteration in the size and shape of the alveolar epithelial cells which often showed a transition to a cuboidal or even a columnar type. Some alveoli showed proliferation of these enlarged epithelial cells which projected into the alveolar spaces and in some areas filled them so completely that the groups of alveoli exhibiting these features presented an appearance suggesting a granulomatous infiltration or an adenomatous growth. In 2 of the 3 cases an incomplete hyaline membrane lined the walls of some of the alveoli. In all 3 cases there was a cellular exudate in the alveolar septa consisting of lymphocytes, plasma cells, eosinophils, and histiocytes. There was no significant polymorphonuclear exudate either in the interstitial tissues or in the alveolar spaces. In none of the lungs were there any specific features of tuberculosis or sarcoidosis, but in one case (*Case 1*) tuberculous follicles were found in sections of the mediastinal lymph nodes. As this patient had a negative Mantoux reaction to 1.0 mg. of Old Tuberculin, the possibility that she might be suffering from sarcoidosis was carefully considered. In the absence of any other clinical or pathologic support for this diagnosis, it was discarded in favor of diffuse interstitial fibrosis of the lungs.

In 2 of the 3 cases, the smaller pulmonary arteries and arterioles showed considerable fibrous thickening of the subintimal connective tissue, and in all 3 some proliferation of elastic tissue in the vessel walls was observed, particularly in the media. Cor pulmonale was confirmed at autopsy in all 3 cases.

Pathologic Features of Previously Recorded Cases

The pathologic features in previously recorded cases of diffuse interstitial fibrosis differ in very few respects from those in the present series. The macroscopic changes in the lungs and pleura were virtually identical, but the firm and

rubbery consistence of the lungs was apparently not so striking in the more rapidly fatal "acute" cases, such as those described by Hamman and Rich (1). The various writers agreed that the thickening of the alveolar septa was in the main due to fibrous tissue, but some believed that vascular congestion, proliferation of the capillaries, and the interstitial edema also played a part in certain cases. There was little disagreement regarding the changes in the cellular lining of the alveoli. The acidophilic hyaline membrane lining the alveolar walls, which appeared to be a particularly distinctive feature, was noted in more than half of the recorded cases. The absence of any significant inflammatory exudate within the alveoli was emphasized by all writers.

In contrast to the present series, the bronchioles in many of the previously recorded cases were reported to be abnormal, the changes reported including squamous metaplasia of the epithelium and exudate or fibrosis, either within or around the bronchiolar walls. The arteriolar changes noted in the present series were identical to those recorded in earlier accounts.

In most of the cases the absence of acid-alcohol-fast bacilli in stained sections and the failure to isolate any other microorganisms, including viruses, by culture or animal inoculation were specifically mentioned.

Treatment

Many drugs, differing widely in their pharmacologic properties, have been used in the treatment of diffuse interstitial fibrosis of the lungs. On the assumption that the condition might have an infective basis, sulfonamides, penicillin, chlortetracycline, oxytetracycline, streptomycin, and isoniazid have been tried, but entirely without success (7, 10, 11, 13, 14, 20, 22–24). Penicillin was given without benefit in *Cases 1* and *2* in the present series. The patient of *Case 1* also received streptomycin, PAS, and isoniazid empirically, with equal lack of success. Calciferol, potassium iodide, and various antihistamine drugs, which were used in three cases (10, 13, 14), had no influence on the course of the illness.

Possible Adverse Influence of Cortisone or Corticotropin

Corticotropin and cortisone have had a variable effect. In four cases (2, 18–20) they promoted definite clinical improvement. In three of these cases (2, 18, 19) the roentgenographic opacities cleared considerably or completely, but in the fourth case (20) they did not alter. In two of the patients who showed both clinical and roentgenographic improvement (18, 19), a rapidly fatal deterioration set in a few days after withdrawal of hormonal therapy, which had been given for two months in one case (18) and one month in the other (19). The patient who showed only clinical improvement (20) maintained this for only two weeks and entered upon a fatal relapse while still receiving cortisone in a dosage of 200 mg. per day. The other patient who improved both clinically and roentgenographically (2) was still alive after seventeen months of cortisone therapy, the dosage having been gradually reduced from 200 mg. to 40 mg. per day. As the diagnosis in this case was confirmed by lung biopsy, the efficacy of cortisone in prolonging the patient's life and in suppressing his symptoms can scarcely be denied.

In four other cases (19, 24), although hormonal therapy did not produce any objective clinical or roentgenographic improvement, the patients "felt better." In two of these cases (24) the tempo of the illness was not appreciably altered, but in the other two (19) a dramatic fatal deterioration followed withdrawal of the drugs.

Although much more information regarding the effects of cortisone and corticotropin on diffuse interstitial fibrosis of the lungs is still required, three tentative conclusions can be drawn from the experience to date: (*a*) only a small proportion of patients obtain a useful degree of symptomatic and roentgenographic improvement; (*b*) those who do so are liable to an acute relapse whenever treatment is stopped, and there is thus a strong case for maintaining treatment throughout life; and (*c*) even when there is no response to treatment, its withdrawal may be followed by rapid clinical deterioration.

DISCUSSION

The mysterious nature of diffuse interstitial fibrosis of the lungs is invariably alluded to by all writers who have studied it. From the clinical standpoint, the position has now been reached in which the condition can be recognized with a fair measure of confidence during life, when a patient with progressive exertional dyspnea is found to have fully developed finger clubbing and diffuse patchy and/or striate roentgenographic opacities throughout both lungs. The pathology, however, seems to constitute a problem within a problem, and there is little agreement even on the sequence and significance of the histologic changes. In the two instances in which it was possible to compare the histologic appearances at autopsy with those noted at biopsy six months previously (19, 20), the main difference in the later sections was that the changes in the alveolar epithelium were less marked while the interstitial fibrosis was more diffuse and advanced. Other workers (4) claim to have demonstrated the origin of the fibrosis in the hyaline membrane lining the alveoli, from which they believe it later extends into the interstitial tissues of the lungs. Regarding the controversy whether the thickening of the alveolar septa is mainly due to fibrous tissue, to an increase in the size and number of pulmonary capillaries (9), or to other factors such as interstitial edema and cellular infiltration, it seems conceivable that the various views are based on different phases of the same disease process.

More fundamental is the disagreement that exists regarding the specificity of the histologic appearances. Many writers believe that similar changes, including the hyaline membrane lining the alveolar spaces, may be found in all forms of interstitial fibrosis whatever its cause, but others (15) take the opposite view and state that the histologic changes are characteristic.

In some of the papers there is speculation regarding the existence of localized forms of the disease and of relatively mild nonprogressive cases, but there is as yet no substantial pathologic proof that the disease can exist in either of these two benign forms.

When the discussion passes to the question of etiology, a certain convergence of view occurs, mainly because the possible causes of the disease are so strictly limited in the present state of our knowledge. There is, for example, virtually

complete agreement that bacterial infection bears no causal relationship to the disease although it may supervene either intercurrently or terminally. There is an equal measure of agreement as to the possibility of a viral infection being responsible for the condition although the logic for this view is perhaps not very securely based. The fact, which many writers have cited, that various types of viral infection can cause localized interstitial fibrosis in the lungs can scarcely be regarded as sufficient grounds for concluding that a group of patients characterized by *diffuse* fibrosis are all suffering from a specific, but as yet unrecognized, viral infection. Nevertheless a viral origin for some of the cases is undoubtedly possible.

Similar *non sequitur* arguments apply when the question of the disease being a manifestation of tissue hypersensitivity is considered in analogy with the so-called collagen diseases which are known to be capable of producing interstitial pulmonary fibrosis. Further points against such an etiologic basis are the invariable restriction of the lesions to the lungs and the absence of the characteristic arteriolar changes. Nevertheless, a relationship to the "collagen diseases" cannot be entirely discounted in view of the close similarity between the histologic changes in the lungs in diffuse interstitial fibrosis and conditions such as scleroderma, disseminated lupus erythematosus, and rheumatoid arthritis (25). Perhaps the most convincing piece of circumstantial evidence in favor of the hypersensitivity theory is the recent observation that hypotensive drugs, such as Apresoline® or the hexamethonium compounds, can produce an illness virtually indistinguishable from disseminated lupus erythematosus in some patients and one closely resembling an acute form of diffuse interstitial fibrosis of the lungs in others (26, 27). If some form of hypersensitivity were in fact responsible for diffuse interstitial fibrosis, it might be possible to explain the strict localization of the disease to the lungs by postulating that the agent to which hypersensitivity is present enters the body via the air passages.

The histologic changes in long-standing cases of pulmonary sarcoidosis may lose their specific features and become practically indistinguishable from those of diffuse interstitial fibrosis. Unless evidence of sarcoidosis is found in the other organs, such cases will probably be accepted, rightly or wrongly, as examples of the type of fibrosis described by Hamman and Rich (1), although of a more chronic character. At the moment, however, it serves no useful purpose to postulate that sarcoidosis, itself a disease of unknown origin, may be an etiologic factor in diffuse interstitial fibrosis.

According to Hayward (28), interstitial pulmonary edema of a subacute or chronic type occurring in diseases such as uremia, polyarteritis nodosa, malignant hypertension, and rheumatic fever may undergo organization and produce interstitial fibrosis resembling that under review. A primary cause for pulmonary edema was not recognized in any of the recorded cases of diffuse interstitial fibrosis of the lungs, but the possibility of such a basis for the condition cannot wholly be denied on that account.

In the end one is forced to the conclusion that, although in diffuse interstitial fibrosis of the lungs one may be dealing with a single pathologic end state, it is

brought about by a variety of agents acting possibly in a variety of ways. It is conceivable that viral infections, hypersensitivity states, and various chemical agents can all produce in the lungs epithelial damage and dysplasia followed by interstitial fibrosis. The resulting process, although varying greatly in its rate of progression, may eventually result in virtual strangulation of the parenchymal tissue by the fibrous reaction.

SUMMARY

Three more cases of diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome) are described, 2 in elderly women and the third in a thirteen-year-old girl.

These cases demonstrated the typical clinical features of progressive exertional dyspnea and cyanosis. The patients all showed clubbing of the fingers, which appears to be a useful point in distinguishing the condition from sarcoidosis. In previously recorded cases, finger-clubbing was often present, but its diagnostic value was not specifically mentioned by any writer.

Death was due in each case to respiratory insufficiency, precipitated in one by pulmonary arterial thrombosis and in another by acute tracheobronchitis; in all 3 patients, cor pulmonale was present. The autopsy findings differed in no essential respect from those in cases previously recorded. Macroscopically, the lungs were grossly fibrotic; the histologic features were those of interstitial fibrosis and of an increase in the size and shape of the alveolar epithelial cells, with the formation of a hyaline membrane in 2 cases.

The clinical, roentgenographic, and pathologic features of the other 36 cases so far recorded have been summarized and analyzed. No progress has been made toward discovering the cause of the condition since Hamman and Rich described the first case twelve years ago. The wide variation in the duration of the illness, from a few weeks to several years, suggests that it may not be a distinct pathologic entity but the end result of several different processes, which may include viral infections and hypersensitivity to drugs or other agents so far unidentified.

In a few cases, cortisone and corticotropin have promoted symptomatic and occasionally even roentgenographic improvement, but only one patient so treated is reported to be alive. There appears to be a serious danger of a lethal exacerbation of the disease when hormonal therapy is withdrawn, even if it has apparently had no beneficial effect.

SUMARIO

Fibrosis Intersticial Difusa de los Pulmones (Síndrome de Hamman-Rich): Reseña con Tres Casos Nuevos

Describense 3 casos más de fibrosis intersticial difusa de los pulmones (síndrome de Hamman-Rich), 2 en ancianas y el tercero en niña de trece años.

Estos casos mostraron las típicas características clínicas de disnea y cianosis progresivas de esfuerzo. Todas las enfermas tenían dedos hipocráticos, lo cual parece ser un punto útil para diferenciar la dolencia de la sarcoidosis. En los casos comunicados anteriormente, hubo a menudo dedos hipocráticos, pero sin que ningún autor mencionara específicamente su valor diagnóstico.

En todos los casos, la muerte se debió a insuficiencia respiratoria, precipitada en uno por trombosis pulmoarterial y en otro por tráqueobronquitis aguda; en las 3 enfermas había corazon pulmonar. Los hallazgos antópsicos no discreparon en ningún punto esencial de los de los casos descritos antes. Macroscópicamente, los pulmones estaban fibrosados; las características histológicas eran las de la fibrosis intersticial y de aumento del tamaño y de la forma de las células epiteliales de los alvéolos, con formación de membrana hialina en 2 casos.

Se sumarizan y analizan las características clínicas, radiográficas e histopatológicas de los otros 36 casos publicados hasta ahora. No ha habido adelanto en lo tocante a descubrir la causa del trastorno desde que Hamman y Rich describieran el primer caso hace doce años. La gran variación en la duración de la enfermedad, de unas pocas semanas a varios años, sugiere que tal vez no se trate de una entidad morbosa independiente, sino del resultado terminal de varios procesos distintos, que pueden comprender infecciones virales e hipersensibilidad a drogas u otros agentes sin identificar hasta ahora.

En algunos casos, la cortisona y la corticotropina han estimulado mejoría sintomática y a veces hasta radiográfica, pero no hay mención más que de una enferma tratada en esa forma que esté viva. Parece que existe grave peligro de exacerbación letal de la dolencia cuando se suspende la hormonoterapia, aunque no haya ejercido aparentemente efecto beneficioso.

RESUME

Sclérose interstitielle diffuse des poumons (Syndrome de Hamman-Rich): Un compte-rendu avec rapport sur trois cas additionnel

Trois autres cas de sclérose interstitielle diffuse du poumon (Syndrome de Hamman-Rich) sont décrits, deux concernant des femme âgées et le troisième chez une petite fille de 13 ans.

Ces cas présentaient les aspects cliniques spécifiques de la dyspnée d'effort progressive et de la cyanose. Tous les malades avaient le signe du doigt hippocratique qui paraît utile pour distinguer cette affection de la sarcoïdose. Dans les cas antérieurement publiés, les doigts hippocratiques figuraient souvent, mais aucun des auteurs ne faisait spécifiquement mention de leur valeur diagnostique.

Dans chaque cas, la mort était due à l'insuffisance respiratoire; une thrombose artérielle avait précipité la mort dans un cas et une trachéo-bronchite dans l'autre. Le coeur pulmonaire existait chez les trois malades. Les résultats de l'autopsie ne différaient pas de façon essentielle de ceux des cas rapportés antérieurement. Macroscopiquement, les poumons apparaissaient largement scléreux; les caractéristiques histologiques étaient ceux de la sclérose interstitielle avec un accroissement des dimensions et modification de la forme des cellules alvéolaire et, dans 2 cas, formation d'une membrane hyaline.

Les aspects cliniques, radiologiques et pathologiques des 36 autres cas enregistrés jusqu'à présent sont résumés et analysés. Aucun progrès n'a été fait quant à la découverte de cause de l'affection depuis la description originale de Hamman-Rich il y a douze ans. La grande variation observée dans la durée de la maladie, qui s'étend de quelques semaines à plusieurs années, suggère qu'elle pourrait ne pas être une entité pathologique distincte, mais le résultat ultime de divers processus pouvant comprendre des infections virales et l'hypersensibilité à certains médicaments ou autres agents non identifiés jusqu'à présent.

Dans quelques cas, la cortisone et l'hormone corticotrope ont entraîné une amélioration fonctionnelle et même parfois radiologique, mais il n'y a qu'un seul des malades ainsi traités qui soit encore vivant. Il paraît exister un grave danger d'exacerbation de l'affection quand le traitement hormonal est suspendu, même lorsqu'il n'a pas paru exercer d'effet favorable.

Acknowledgment

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SIMPLE TESTS OF RESPIRATORY FUNCTION USING A DIRECT-WRITING EAR OXIMETER[1]

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INTRODUCTION

A number of pulmonary function tests requiring an ear oximeter have been devised. The oximeter will record continuously the percentage of oxygen saturation of blood in the ear; this will correspond closely to the arterial oxygen saturation if the ear capillaries are dilated by warmth (1), application of a rubefacient ointment, or histamine iontophoresis.

When a subject breathes approximately 100 per cent oxygen, the arterial oxygen saturation rises steeply at first, then more gradually, and finally levels out to form a plateau (figure 1). Some tests have involved measurement of the time or a fraction of the time which had elapsed from the beginning of oxygen breathing until the arterial oxygen saturation ceased to rise further (2–8). In other tests the subject was first made anoxic by breathing a low oxygen mixture and was then given air or 100 per cent oxygen to breathe, and resaturation times were estimated (2, 3, 9, 10). Kossman and Briller (11) gave fully oxygenated patients a low oxygen mixture to breathe and observed the fall of the arterial oxygen saturation and the speed of the return to normal. Ferguson, Finlayson, and Hilliard (12) measured the rate of decline of oxygen saturation on changing from breathing oxygen to breathing air.

Changes in arterial oxygen saturation during hyperventilation have been recorded by means of an oximeter in normal subjects and in patients with congenital heart disease (13). Oximeter readings have been made during exercise in normal subjects (4, 13), in patients with congenital heart disease (4, 12, 13), and in some patients with pulmonary disease (12, 14).

In the present investigation the "oxygen saturation time" has been found to be unreliable as a diagnostic test and as a test of respiratory function. The "oxygen desaturation time," on the other hand, is shown to be valuable in distinguishing patients with emphysema from patients with other pulmonary disease. A rise in arterial oxygen saturation has been shown to accompany voluntary hyperventilation. Exercise, even in normal subjects, results in a fall in arterial oxygen saturation and this fall may be considerable in patients with pulmonary disease. The tests described, employing the ear oximeter, are simple to perform and cause the patient little discomfort.

METHODS

The direct-writing ear oximeter has been described by Paul (15, 16). The aluminum earpiece contains a lamp operated by an interrupted direct current, two filters, and two selenium photo cells. One filter transmits light at the 650 mu band (red), the other at 800 mu

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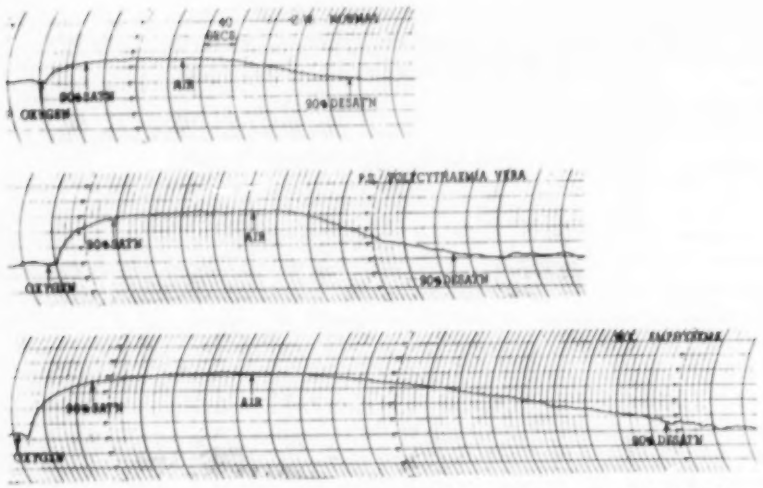

FIG. 1. Saturation and desaturation curves (to be read from left to right).

(infrared). The alternating voltages produced by light passing through the pinna to the photo cells operate two meters. A zero correction for light absorption by non-hemoglobin substances in the light path (skin, subcutaneous tissue, cartilage) is effected by application of pressure by a pneumatic capsule to exclude blood. After release of pressure the two meter readings indicate light absorption by the blood in the ear in the two color regions. The output of the red photo cell varies with the degree of oxygenation of the blood and with changes in total hemoglobin, while the output of the infrared cell varies only with total hemoglobin. An automatic balancing system computes the ratio of the output of the two filter-photo cell systems and operates an ink-writing Esterline-Angus milliameter. The changes in photo cell output caused by fluctuations in total hemoglobin content are canceled in the computer, and the final record indicates only changes in the degree of oxygenation of the blood.

A small amount of rubefacient ointment (Trafuril®)[1] was applied for five minutes to the right pinna before the earpiece was placed in position. For the oximeter tests the subject breathed 99.8 per cent oxygen from either a Boothby-type mask or from a 9 liter Collins respirometer. When the latter was used, a respiratory tracing was obtained from which the oxygen consumption and minute volume could be calculated. The concentration of oxygen in the spirometer decreased a small amount because of the admixture of nitrogen washed out from the lungs. The patient breathed from the spirometer for only a few minutes and the oximeter tracing leveled off in the same way as it did when the oxygen was supplied by the Boothby-type mask. After a few seconds (mouth-ear time) the arterial oxygen saturation began to rise and after a variable period a plateau was reached. The subject now breathed room air, and gradually the arterial oxygen saturation fell until the original steady state was reached.

The rate of arterial oxygen saturation and desaturation was expressed as the "90 per cent saturation time" and the "90 per cent desaturation time." The 90 per cent saturation time was measured as time in seconds from the beginning of oxygen breathing until the arterial oxygen saturation had risen 90 per cent of the way to the steady state (figure 1). Similarly the 90 per cent desaturation time was the time in seconds from the cessation of

[1] Tetrahydrofurfuryl nicotinic acid ester.

oxygen breathing until the arterial oxygen saturation had fallen 90 per cent of the way to the steady state (figure 1). At the beginning of the investigation the 70 and 80 per cent saturation and desaturation times were also calculated. It soon became apparent that the best distinction between normal subjects and the patients with emphysema was obtained when the 90 per cent value was used.

The effect of hyperventilation on the arterial oxygen saturation was recorded during the measurement of the maximal breathing capacity.

A standard exercise test was carried out by having the subject step up and down a 20-centimeter step thirty times in one minute.

It was possible to express the results in arterial oxygen saturation per cent after the recording paper had been calibrated by making simultaneous ear oximeter records and oxygen estimations on arterial blood.

Respiratory function tests were performed on all but 4 of the patients with pulmonary disease. With the patient at rest in the supine position, a spirogram was recorded using a Collins respirometer. From this tracing the respiratory rate, tidal volume, minute volume, inspiratory capacity, expiratory reserve volume, and vital capacity were calculated. The form of the spirogram during quiet breathing, during rapid breathing, and during the vital capacity estimations was analyzed in accordance with the principles of Cournand, Richards, and Darling (17). The maximal breathing capacity was recorded with the patient erect, and the expired air was collected in a Douglas bag and measured in a gas meter. All volumes were corrected to 37°C. saturated with water vapor at prevailing barometric pressure. The formulas described by Baldwin, Cournand, and Richards (18) were used to calculate the standard normal vital capacity and maximal breathing capacity.

MATERIAL

Oximeter tests were performed on 65 subjects who were divided into five groups. The numbers, ages, and sex distributions are shown in table 1.

Group I. Normal subjects who were members of the hospital staff and showed no abnormality as determined by history and roentgenographic examination

Group II. Hospital patients who showed no evidence of pulmonary abnormality by history, on physical examination, and on roentgenography

Group III. Hospital patients in whom there was pulmonary abnormality of an insignificant degree or in whom pulmonary insufficiency was suspected but in whom the pulmonary function studies were normal (*see* table 4)

Group IV. Patients with definite pulmonary disease in whom examination, roent-

TABLE 1

NUMBERS, AGES, AND SEX DISTRIBUTION OF THE SIXTY-FIVE SUBJECTS ON WHOM OXIMETER TESTS WERE PERFORMED

Group	Type	Males	Females	Total	Age Range	Mean Age
					years	*years*
I	Normal subjects	8	9	17	22–36	28
II	Hospital patients with normal lungs	11	0	11	19–66	41
III	Patients with slight pulmonary abnormalities unlikely to interfere with function	3	3	6	26–71	42
Total "normal"		22	12	34	19–71	35
IV	Patients with pulmonary abnormalities other than emphysema	10	5	15	25–64	42
V	Patients with emphysema	15	1	16	29–70	52

TABLE 2

TYPES OF PULMONARY DISEASE PRESENT IN GROUPS IV AND V

Group	Diagnosis	Number of Patients
IV	Pulmonary fibrosis	3
	Bronchiectasis	4
	Secondary carcinoma	1
	Pneumonectomy with bronchial obstruction	1
	Calcified tuberculosis	1
	? Sarcoidosis, active	1
	Pulmonary congestion	1
	Hemosiderosis	1
	Mild silicosis	1
	Poliomyelitis with intercostal and diaphragmatic weakness	1
V	Emphysema	14
	Emphysema and pulmonary fibrosis	2
Total		31

genography, and respiratory function tests failed to reveal the presence of emphysema

Group V. Patients in whom the diagnosis of diffuse obstructive emphysema (with or without pulmonary fibrosis) had been established by clinical and roentgenographic examination and by respiratory function tests.

Groups I, II, and *III* have been used as one group to represent subjects with normal pulmonary function.

The types of pulmonary disease present in *Groups IV* and *V* are shown in table 2.

RESULTS

The 90 Per Cent Saturation Time

One hundred and thirty-three oximeter tests were performed on 65 subjects. The results are shown graphically in figure 2. It will be noted that the normal subjects (*Group I*) showed 90 per cent oxygen saturation times closely grouped around the mean of 43.4 seconds (S.D. ± 13.4 secs.). The other groups showed a wider scattering of results. The mean 90 per cent saturation time of *Group II* was 67.7 seconds (S.D. ± 25.8 secs.); of *Group III*, 65.4 seconds (S.D. ± 25.4 secs.); of *Group IV*, 72.2 seconds (S.D. ± 25.0 secs.); and of *Group V*, 105 seconds (S.D. ± 37.6 secs.).

The mean 90 per cent oxygen saturation time of the patients with emphysema was greater than that of the other groups. This prolongation of the saturation time was of little diagnostic value in the individual case because of the considerable overlap of the 90 per cent saturation times of all groups (figure 2).

Lack of reproducibility also detracted from the usefulness of this test. A statistical analysis of 64 double tests showed the standard deviation of a single measurement to be 18.5 seconds, or 25.8 per cent of the average of these 128 tests.

It was hoped that better differentiation and reproducibility might be obtained if the 90 per cent saturation time were related to oxygen consumption and minute

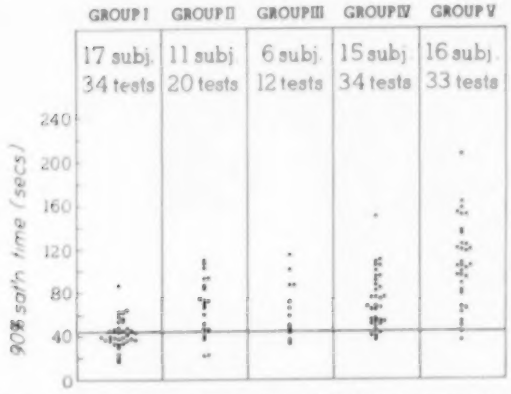

FIG. 2. Ninety per cent saturation times. The solid line indicates the mean of the tests of *Group I*. *Group I:* normal subjects; *Group II:* hospital patients without evidence of pulmonary abnormality; *Group III:* hospital patients with insignificant pulmonary abnormality; *Group IV:* patients with definite pulmonary abnormality but without emphysema; *Group V:* patients with emphysema.

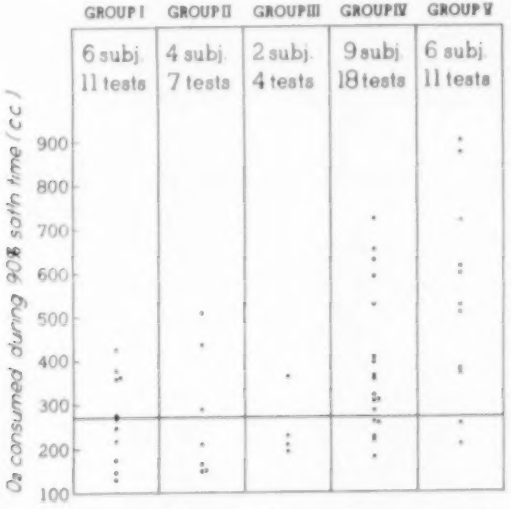

FIG. 3. Oxygen consumption during the 90 per cent saturation time. The solid line indicates the mean of the tests of *Group I*.

volume. The 90 per cent saturation time was measured and the oxygen consumption and volume of gas (oxygen) breathed during this time were calculated. The results are illustrated in figures 3 and 4, and again there is no clear-cut differentiation between normal and abnormal groups. Twenty-four double estimations of oxygen consumption during the 90 per cent saturation time were analyzed

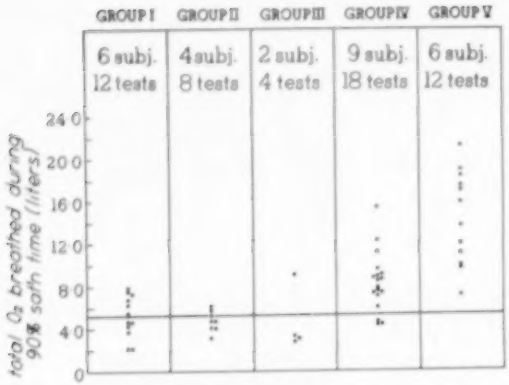

FIG. 4. Total gas (oxygen) breathed during 90 per cent saturation time. The solid line indicates the mean of the tests of *Group I*.

and the standard deviation of a single measurement was 117 ml., or 33 per cent of the average of these 48 tests. Similarly, statistical examination of 27 double estimations of oxygen breathed during the 90 per cent saturation time revealed the standard deviation of a single measurement to be 2.78 liters, or 34 per cent of the average of these 54 tests. These tests were thus of no greater value than the measurement of 90 per cent saturation time alone.

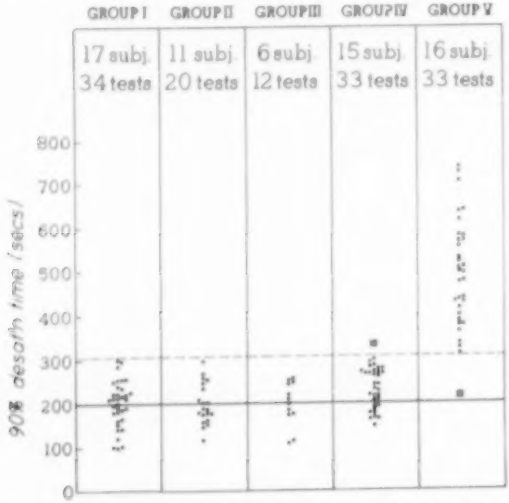

FIG. 5. Ninety per cent desaturation times. The solid line indicates the mean of the tests of *Group I*. The broken line is at 300 seconds and divides the tests of *Group V* from those of the other groups. Tests marked ⊡ are the exceptions described in the text.

The 90 Per Cent Desaturation Time

One hundred and thirty-two tests were performed on 65 subjects. The results are shown graphically in figure 5 and recorded in tables 3 and 4. The mean 90 per cent desaturation time of *Group I* was 201.4 seconds (S.D. ± 52.1 secs.); of *Group II*, 198.5 seconds (S.D. ± 46.0 secs.); of *Group III*, 196.8 seconds (S.D. ± 48.8 secs.); of *Group IV*, 232.9 seconds (S.D. ± 46.3 secs.); and of *Group V*, 487.6 seconds (S.D. ± 123.0 secs.). The average 90 per cent desaturation times of the subjects with no significant pulmonary disease (*Groups I*, *II*, and *III*) were almost identical, and the mean 90 per cent desaturation time of the patients who had pulmonary disease but not emphysema (*Group IV*) was only slightly greater. The average 90 per cent desaturation time of those with emphysema (*Group V*) was more than double that of any of the other groups. With

TABLE 3

NINETY PER CENT DESATURATION TIME AND EFFECT OF HYPERVENTILATION AND EXERCISE ON ARTERIAL OXYGEN SATURATION IN GROUPS I AND II

Group	Patient	Age	Sex	90% Desaturation Time	Hyperventilation Rise in Arterial Oxygen Saturation	Hyperventilation Index	Exercise Fall in Arterial Oxygen Saturation
		years		*seconds*	*per cent*	*per cent*	*per cent*
I	B. K.	27	F	215	—	—	—
	W. S.	28	M	292	—	—	—
	L. W.	25	F	167	1.4	100.0	1.2
	B. P.	36	M	153	3.5	100.0	1.9
	T. N.	26	M	161	1.9	36.4	3.0
	R. G.	32	M	169	2.2	75.0	2.4
	H. W.	31	M	194	1.4	61.5	2.5
	J. P.	28	F	110	2.7	100.0	0
	E. C.	25	M	277	1.4	54.5	2.4
	C. W.	30	M	197	0.5	25.0	0.7
	S. D.	23	F	245	1.4	46.2	2.6
	J. B.	26	F	217	0.9	60.0	2.2
	J. A.	28	F	184	0.7	30.8	3.8
	M. F.	36	F	201	1.4	90.7	0.7
	M. G.	22	F	214	0.5	39.8	0.7
	M. K.	28	F	230	1.2	100.0	1.2
	E. K.	24	M	254	1.4	61.5	3.2
II	V. N.	52	M	156	—	—	—
	G. B.	48	M	175	—	—	—
	M. F.	22	M	282	—	—	—
	W. K.	19	M	193	1.9	57.2	1.4
	B. W.	54	M	130	1.4	72.7	0
	B. C.	43	M	249	1.2	58.3	0
	W. C.	44	M	164	1.0	40.0	1.1
	D. C.	35	M	180	1.4	61.7	1.1
	V. H.	42	M	180	—	—	—
	G. G.	27	M	209	—	—	—
	W. C.	66	M	246	—	—	—

TABLE 4

NINETY PER CENT DESATURATION TIME AND THE EFFECT OF HYPERVENTILATION AND EXERCISE ON ARTERIAL OXYGEN SATURATION COMPARED WITH TESTS OF RESPIRATORY FUNCTION

Group	Patient	Diagnosis	Age	Sex	V.C.	M.B.C.	Air Trapping 0 to +++	90% Desaturation Time	Hyperventilation Rise in Arterial Oxygen Saturation	Hyperventilation Index	Exercise Fall in Arterial Oxygen Saturation
			years		*per cent*	*per cent*		*seconds*	*per cent*	*per cent*	*per cent*
III	P. S.	Polycythemia vera	42	F	82.6	86.8	+	247	2.2	30	—
	A. P.	Sarcoid treated; lungs 'normal'	26	F	110.0	87.5	0	232	1.4	50	3.4
	J. R.	Polycythemia; meningioma; pulmonary stenosis	39	M	78.0	97.8	0	180	4.0	54	0
	H. Z.	Acromegaly	39	F	118.0	89.5	0	185	1.9	47	2.9
	W. H.	Polycythemia	71	M	125.0	98.5	0	211	2.9	32	3.2
IV	J. M.	Pulmonary fibrosis (idiopathic)	64	M	77.5	87.2	0	291	—	—	6.0
	J. B.	Pulmonary fibrosis (sarcoid)	38	F	58.0	91.5	+	222	1.0	37	6.0
	F. G.	Pulmonary fibrosis (sarcoid)	40	M	69.2	51.2	+	247	0.9	13	3.7
	W. M.	Bronchiectasis; destroyed left lung	53	M	58.4	49.5	+	203	2.6	41	—
	W. F.	Bronchiectasis; anasarca; peripheral neuritis; obesity	32	M	50.0	25.3	+	270	—	—	—
		After three weeks edema decreased			52.7	23.2	+	312	3.9	34	—
	R. D.	Bronchiectasis, left upper lobe	32	F	88.2	83.5	0	217	0.7	40	0
	A. R.	Pulmonary metastases; pleural effusions	46	F	33.8	52.0	+	211	2.1	30	15.6
	S. H.	Right pneumonectomy; stenosis, left main bronchus	47	M	31.2	34.0	+ +	269	1.0	8	7.2
	A. C.	Scattered calcification; inactive tuberculosis	46	M	89.5	99.0	0	240	1.7	17	0
	W. W.	Silicosis; bronchospasm	54	M	86.0	35.4	+	170	0.7	12	2.1

	J. H.	Congestive heart failure	45	M	59.0	69.8	0	257	4.6	36	0
	W. M.	Mitral stenosis; hemosiderosis	28	M	71.0	63.7	+	168	1.4	53	1.8
	I. C.	Diffuse pulmonary mottling; ? sarcoid	45	F	93.5	80.7	+	237	3.1	46	2.6
	J. H.	Poliomyelitis, intercostals diaphragm	28	F	19.3	—	0	172	1.9	47	—
V	A. H.	Emphysema	52	M	65.5	24.3	+ + +	402	0.7	5	16.0
	B. H.	Emphysema	39	M	61.0	25.6	+ + +	493	0.7	9	6.7
	C. T.	Emphysema	57	M	52.3	26.8	+ + +	544	3.0	19	—
	K. Sl.	Emphysema; pulmonary fibrosis	59	M	36.9	27.0	+ + +	575	—	—	—
	K. Sp.	Emphysema; pulmonary fibrosis	45	M	64.4	31.4	+ + +	380	2.9	25	5.8
	W. K.	Emphysema	60	M	72.5	32.0	+ + +	554	1.2	13	1.7
	O. W.	Emphysema	66	M	67.0	33.4	+ + +	520	0.7	24	2.2
	C. G.	Emphysema	63	M	68.8	33.5	+ + +	507	7.5	49	17.0
	W. H.	Emphysema	70	M	49.0	34.6	+ + +	452	4.1	21	10.3
	C. M.	Emphysema	43	M	78.2	43.6	+ + +	685	1.8	20	3.4
	E. D.	Emphysema	52	M	75.6	51.5	+ +	326	5.4	60	4.9
	G. W.	Emphysema	55	M	72.6	58.3	+ +	569	1.0	7	6.2
	W. G.	Emphysema	32	M	114.0	59.9	+ +	713	4.0	56	1.8
	C. W.	? Emphysema	29	M	100.0	110.0	+	466	1.8	21	1.3

only one exception (in *Group IV*), the 90 per cent desaturation times of subjects in *Groups I, II, III,* and *IV*, that is, subjects without emphysema, were less than 300 seconds. In *Group V*, with emphysema, all of the tests, except one, showed a 90 per cent desaturation time of more than 300 seconds.

Statistical analysis of 62 double tests showed that the standard deviation of a single determination was 35 seconds, or 12.5 per cent of the average of these 124 tests. Thus the test was reproducible with reasonable accuracy.

The patient (*W.F.*) without emphysema, but in whom the 90 per cent desaturation time was more than 300 seconds, was a diagnostic problem. He was a 32-year-old alcoholic, bedridden with peripheral neuritis. He was admitted with anasarca, and his skin showed a mottled cyanosis. Bullae were present at the apex of the left lung, and the left lower lobe was collapsed and bronchiectatic. The patient's vital capacity was 50 per cent of standard normal and the maximal breathing capacity was 25.3 per cent of normal, but the spirogram showed no air trapping. The 90 per cent desaturation times were 272 and 269 seconds. After the edema and ascites had decreased, there was no significant change in the vital capacity or in the maximal breathing capacity, and the 90 per cent desaturation times were 290 and 334 seconds. Possibly in this last test oxygen might have collected in the bullae in the apex of the left lung and was only slowly washed out from this area, resulting in the slightly prolonged 90 per cent desaturation time.

The exceptional patient with emphysema was *K.S.*, a 42-year-old white male with gross diffuse pulmonary fibrosis and emphysema and many large and small bullae in both lungs. His vital capacity was 64.4 per cent of the average normal, the maximal breathing capacity was 31.4 per cent, and the spirogram showed severe air trapping. The 90 per cent desaturation times were 383 seconds, 213 seconds, and 378 seconds. The normal desaturation in the second test is difficult to explain, but might be due to bullae not filling with oxygen.

A case of some interest was that of *C.W.*, a 29-year-old man, who had had severe asthma for a year. In spite of clinical evidence of mild emphysema, the maximal breathing capacity was 110 per cent of standard normal. The 90 per cent desaturation time was abnormal (466 seconds). Possibly a prolonged 90 per cent desaturation time is a sensitive indication of early emphysema.

The Effect of Hyperventilation on Arterial Oxygen Saturation

In every case there was a rise in arterial oxygen saturation in response to hyperventilation (tables 3 and 4). Two or more tests were done on most subjects but only the highest rise that occurred in each case was recorded in the tables.

In the 25 subjects in *Groups I, II,* and *III*, the mean rise was 1.7 saturation per cent with a variation from 0.5 to 4.0 saturation per cent, the highest figure being obtained in a patient with polycythemia. Three subjects showed a rise of less than 1.0 saturation per cent. In some cases the rise was followed by a fall below the resting level and then a gradual return to normal. Examples of normal response to hyperventilation are shown in figure 6.

In the 13 patients of *Group IV* the mean rise was 2.0 saturation per cent, with a variation from 0.7 to 4.6 saturation per cent. The highest figure was ob-

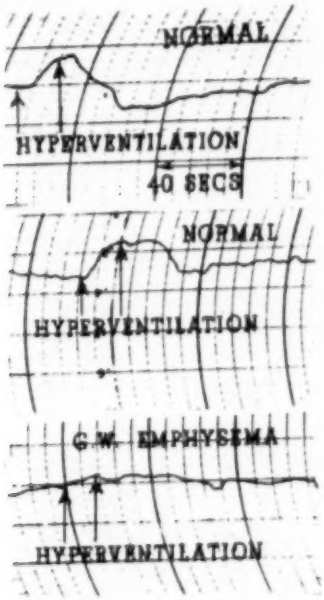

FIG. 6. The effect of hyperventilation on the arterial oxygen saturation (to be read from left to right).

tained in a patient with congestive cardiac failure. Three subjects showed a rise of less than 1.0 saturation per cent.

In the 13 patients of *Group V*, the mean rise was 2.7 saturation per cent with a variation from 0.7 to 7.5 saturation per cent. The greatest rise was observed in a patient with emphysema and secondary polycythemia. Three patients showed a rise of less than 1.0 saturation per cent.

More significant differences appeared when the rise in arterial oxygen saturation during hyperventilation was compared with the rise in arterial oxygen saturation when the patient breathed oxygen. The rise during hyperventilation expressed as a percentage of the rise caused by breathing oxygen was termed the "hyperventilation index." In *Groups I*, *II*, and *III*, the hyperventilation index varied from 25 to 100 per cent, with a mean of 59 per cent; only one of the 25 subjects showed a figure of less than 30 per cent. In *Group IV* the variation was 7.7 to 53.3 per cent with a mean of 32 per cent, and 4 of the 13 subjects showed values of less than 30 per cent. In *Group V* the variation was 5.3 to 60 per cent with a mean of 25 per cent, and 10 of the 13 patients showed values of less than 30 per cent. Thus, in patients with emphysema the arterial oxygen saturation rose relatively less on hyperventilation than on breathing oxygen.

The Effect of Exercise on Arterial Oxygen Saturation

In *Groups I*, *II*, and *III*, exercise tests were done on 24 subjects. There was a fall of arterial oxygen saturation in 20 and no fall in 4 of these subjects. The mean

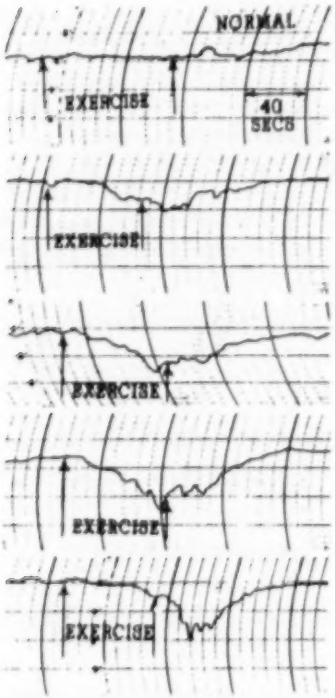

FIG. 7. The effect of a one-minute step exercise on the arterial oxygen saturation of normal subjects (to be read from left to right).

fall of these 20 subjects was 2.1 saturation per cent. The lowest fall of arterial oxygen saturation during the test was 3.8 saturation per cent. The variations of normal are shown in figure 7.

In *Group IV* exercise tests were done on 11 subjects. There was no fall of arterial oxygen saturation in 3, a fall within normal limits in 4 (that is less than 3.8 saturation per cent), and an abnormal fall (greater than 3.8 saturation per cent) in 4 patients. The greatest fall of arterial oxygen saturation was 15.6 saturation per cent. The diagnoses in this last group were idiopathic pulmonary fibrosis; pulmonary fibrosis secondary to sarcoidosis; carcinoma of the breast with pleural effusions and probably lymphangitic carcinoma; and post right pneumonectomy with stenosis of the left main bronchus. In the first 2 patients the defect was probably one of alveolar-capillary block; in the third, a mixed ventilation and diffusion defect; and in the fourth, a difficulty in ventilation. Examples of the results obtained in these patients are shown in figure 8.

In *Group V* exercise tests were done on 12 patients. In 5 patients the fall was within the normal range, but in the remaining 7 the fall of arterial oxygen saturation was abnormal. The lowest fall of arterial oxygen saturation was 17.0 saturation per cent. Examples are shown in figure 8.

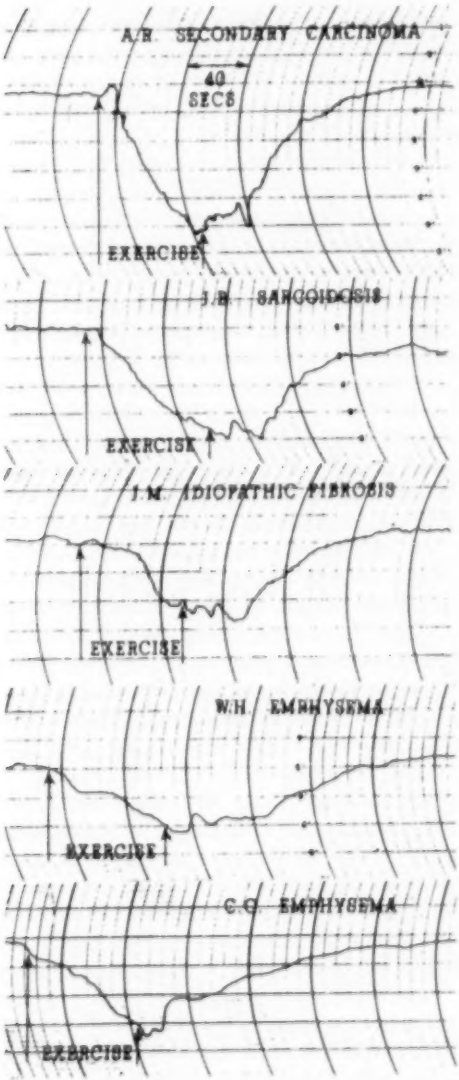

FIG. 8. The effect of a one minute step exercise on the arterial oxygen saturation of patients with pulmonary disease (to be read from left to right). For actual figures *see* table 4.

There was usually little variation when more than one test was done on a subject, but the lowest fall of the arterial oxygen saturation was the one recorded in tables 3 and 4.

In some patients, for example, those with a history of angina pectoris, the

exercise test was not done, and in a few patients the test was not satisfactorily performed. The results of these unsatisfactory tests were not recorded in the tables.

Respiratory function tests were not done on 4 patients, and these patients have been excluded from table 4.

DISCUSSION

The rise of the arterial oxygen saturation when oxygen was breathed by normal subjects has been described by a number of investigators (2–7, 11, 19). Few studies have been made on the rate of rise of arterial oxygen saturation in patients with pulmonary disease. Fowler and Comroe (2) found that patients with pulmonary emphysema showed marked prolongation of the time required to reach maximal arterial oxygen saturation. Saturation curves of a patient with emphysema and a patient with Ayerza's disease were shown in a paper by Fowler and Comroe (3).

Caswell, Milne, and Taplin (10) described 300 resaturation curves on 55 subjects, 8 of whom were normal; the others had impaired cardiac or respiratory function. The patients breathed a low oxygen mixture and, when the arterial oxygen saturation was approximately 85 per cent, the patient was allowed to breathe room air. The resaturation time was the time required for the arterial oxygen saturation to return from approximately 85 per cent to the original value at rest breathing air. They concluded that patients with known respiratory disability had strikingly prolonged resaturation times. There was no description of respiratory function tests or of the types of pulmonary disease investigated. Alexander and Reydman (9) also used a resaturation time, and in 53 per cent of 300 patients the test was abnormal as compared with the results of 55 healthy adults. There was prolongation of the resaturation time especially in patients with emphysema or pulmonary edema.

In the present series, using the 90 per cent saturation time, there was considerable overlap of the results obtained in the normal subjects and those with pulmonary disease. Although, on the average, patients with emphysema had a prolonged 90 per cent saturation time, the test was of little diagnostic value in the individual case. The test was not accurately reproducible. These defects were not corrected when the 90 per cent saturation time was related to oxygen consumption and the volume of oxygen breathed during the test.

Little attention has been directed toward the type of curve produced by the fall of the arterial oxygen saturation from full saturation to the level found when breathing room air. Montgomery and associates (4) recorded the mean desaturation time in 19 normal subjects as 2.8 minutes with a range of 1 to 5 minutes. Ferguson, Finlayson, and Hilliard (12) recorded the rate of decline of oxygen saturation when the patient was changed from breathing oxygen to breathing room air. This was expressed as the time taken for the oximeter reading to fall half way to its stable value with the subject breathing air. This "½ R time" (or 50 per cent desaturation time) was 1.70 ± 0.07 minutes for 41 normal subjects. An examination of unpublished data from this investigation showed a tendency

for the ½ R time to be more prolonged in patients with emphysema and asthma than in patients with other types of pulmonary abnormality. Of 60 patients with pulmonary disease other than emphysema or asthma, 8 (13.3 per cent) had a ½ R time of more than 4 minutes. Of 15 patients with either emphysema or asthma, 8 (53.5 per cent) had a ½ R time of more than 4 minutes.

Zijlstra (14) showed several illustrations of saturation and desaturation curves using the "cyclops" oximeter. In 2 patients with tuberculosis and emphysema he noted the flat descent of the desaturation curves.

Recently, Blair and Hickam (20) stated that, when oxygen was administered for 20 or 30 minutes to an emphysematous patient who exhibited definite arterial oxygen unsaturation when breathing air, the arterial unsaturation often returned only slowly after the oxygen had been discontinued.

In the present study all patients with emphysema had 90 per cent desaturation times of more than 300 seconds. Normal subjects and patients without demonstrable emphysema but with other pulmonary disease had 90 per cent desaturation times of less than 300 seconds. The two exceptional tests have been described. The results of the repeat tests on the same individual agreed reasonably well.

In patients with emphysema it was probable that both the large functional residual capacity and the inefficient gas mixing played a part in the production of the prolonged desaturation time. Because of a large functional residual capacity the patient with emphysema started the test with a larger volume of oxygen in his lungs than a subject without emphysema. In addition, there was a less efficient washout mechanism. Whether oxygen was slowly absorbed from poorly ventilated areas where it remained in high concentration or whether this oxygen enriched the gas mixture to other portions of the lung during the washout period was uncertain.

Other possible factors are unlikely to be important. A rise in arterial oxygen saturation might cause hypoventilation in patients with emphysema and thus increase the "washout time." None of the patients with emphysema showed apnea or hypoventilation clinically, nor was there a decrease in tidal volume or respiratory rate in those cases in which a respiratory tracing had been recorded during the period of oxygen breathing. The final arterial oxygen saturation at the end of the test was approximately the same as that at the beginning of the test before oxygen was given (figure 1). This was indirect evidence that ventilation was not depressed by the breathing of oxygen for a few minutes by the patients with emphysema in this series.

It might be argued that patients with emphysema often have a lower arterial oxygen saturation when breathing air than normal subjects. The rise to full saturation when breathing oxygen would be greater than normal and subsequently it would take longer for the arterial oxygen saturation to fall to the patient's usual level when air breathing was resumed. However, many of the patients with pulmonary disease but without emphysema (*Group IV*) had a rise in arterial oxygen saturation comparable with that of the emphysema cases, but the fall was rapid and the desaturation time normal. Conversely, some of the

emphysematous patients had a relatively small rise in arterial oxygen saturation during the breathing of oxygen, but in spite of this the subsequent fall was prolonged.

It seemed unlikely that the duration of oxygen breathing before the test had any effect on the desaturation time. Many of the patients with emphysema were required to breathe oxygen for a longer period before a satisfactory plateau was established, but other patients who breathed oxygen for a comparable time had normal desaturation times. Conversely, some patients with emphysema achieved a stable arterial oxygen saturation after breathing oxygen for a relatively short period, but still showed a prolonged desaturation time.

Alveolar-capillary block was present in 2 patients, *J.M.*, with idiopathic pulmonary fibrosis, and *J.B.*, with sarcoidosis. Both patients had normal 90 per cent desaturation times. This suggested that abnormality of diffusion across the alveolar-capillary membrane played little part in causing the prolongation of the desaturation time in the patients with emphysema.

Mild bronchospasm was probably unimportant in causing a prolonged desaturation time. In 5 of the patients without emphysema rhonchi were present, but the desaturation times were normal. There were no patients with acute asthma, but without emphysema, in the series.

Patients with severe pulmonary disability but without emphysema had normal desaturation times. For example, *S.H.* had had a right pneumonectomy for bronchial carcinoma 21 months previously and at the time of examination had severe stenosis of his left main bronchus. His vital capacity was 31.2 per cent of normal, his maximal breathing capacity was 34 per cent, and there was an abnormal fall of arterial oxygen saturation during the exercise test. The 90 per cent desaturation time was 269 seconds, which is within the normal range.

Three patients had polycythemia without cardiac or pulmonary abnormality and all had normal respiratory function tests and normal 90 per cent desaturation times. An increase in the circulating erythrocytes will probably not affect the test.

Thus, it seemed probable that the prolongation of the desaturation time was not related to hypoventilation, the presence of a low arterial oxygen saturation, the duration of oxygen breathing before the test was performed, or the presence of alveolar-capillary block, mild bronchospasm, or polycythemia.

Taking all factors into consideration it seemed reasonable to conclude that a 90 per cent desaturation time of more than 300 seconds was characteristic of diffuse obstructive emphysema, with or without pulmonary fibrosis. The degree of prolongation of the desaturation time was not directly related to the severity of the emphysema as judged by clinical disability and the maximal breathing capacity. Except in the advanced case, the clinical and roentgenographic diagnosis of diffuse obstructive emphysema is difficult, especially if pulmonary fibrosis is also present (21, 22). Individual respiratory function tests often give indecisive results, and a group of such tests is required for diagnostic purposes (23, 24). The 90 per cent desaturation time appears to be a simple, reliable, rapid test to confirm the clinical impression of the presence or absence of diffuse obstructive emphysema.

Björk and Hilty (25, 26) showed that in healthy subjects after 20 to 25 seconds' hyperventilation there was no significant increase in the arterial oxygen saturation but there was a definite increase in arterial oxygen tension. Patients with a very poor respiratory reserve were unable to increase the arterial oxygen tension during voluntary hyperventilation. The estimations of arterial oxygen saturation were done on arterial blood.

Houston (27) demonstrated that, in 9 patients breathing a 10.5 per cent oxygen mixture, an increase by half of the resting ventilation resulted in an increase of arterial oxygen saturation, in some cases as high as 10 to 20 per cent. Lambertsen and co-workers (28) caused hyperventilation in 8 normal subjects by having them breathe a gas mixture rich in carbon dioxide but with normal oxygen content. In all there was a small rise in arterial blood oxygen content amounting to usually less than one volume per cent.

Using a Waters-Conley absolute reading oximeter, Van Lingen and Whidborne (13) demonstrated in 25 normal subjects a progressive rise in arterial oxygen saturation during maximal voluntary hyperventilation for one minute. The average increase was 3.5 per cent with a range of 2 to 7 per cent.

In the present series all subjects showed a rise in arterial oxygen saturation during hyperventilation. The rise in normal subjects was somewhat less (mean 1.7 per cent with a range of 0.5 to 4.0 per cent) than that recorded by Van Lingen and Whidborne (13), but in the present investigation hyperventilation was performed for only 15 seconds. There was no definite difference in the rise of those patients with and those without pulmonary disease, except for a tendency for the rise to be higher in the patients with chest disease. This might be due to a lower than normal arterial oxygen saturation in some of the patients with pulmonary disease, and the starting point for hyperventilation was thus on the steeper portion of the oxygen dissociation curve. Under these circumstances a relatively small rise in alveolar oxygen tension would cause a relatively large rise in arterial oxygen saturation. It was considered that the rise of arterial oxygen saturation during hyperventilation might be of more significance if expressed as a percentage of the rise of arterial oxygen saturation caused by breathing oxygen (the hyperventilation index). Only one normal subject showed a hyperventilation index of less than 30 per cent, and when the test was repeated on this subject on another day the hyperventilation index was 38 per cent. Four of the patients with pulmonary disease but without emphysema showed a low hyperventilation index. Two of these had severe pulmonary disease, while the other 2 had not (as judged by clinical status and pulmonary function tests). Severe respiratory disability was present in some of the patients with a normal hyperventilation index. In the patients with emphysema there was no definite correlation between the hyperventilation index and the severity of the disease, as judged by clinical disability and by respiratory function tests. It might be expected that those patients with a low maximal breathing capacity, and thus a limited ability to hyperventilate, would have a low hyperventilation index. Although this was often the case, there were many exceptions.

It may be concluded that the hyperventilation index is of little clinical value in assessing the degree of respiratory disability.

Montgomery and associates (4), using a treadmill, performed exercise tests on 19 normal subjects and by means of an oximeter showed that the arterial oxygen saturation fell, on the average, 0.3 per cent and that there was a range of 0 to 2.0 per cent. Van Lingen and Whidborne (13) used a step test similar to that used in the present investigation and they recorded arterial oxygen saturation also with an oximeter. In 25 normal subjects there was a range from an increase of 1 per cent to a decrease of 3 per cent.

Ferguson and co-workers (12) used a step test and showed by oximetry that 39 of 75 patients with pulmonary abnormality had a fall in arterial oxygen saturation. However, examination of unpublished data from this investigation showed that a fall of as little as 1.0 per cent was taken as significant. Zijlstra (14) using a "cyclops" oximeter demonstrated arterial oxygen desaturation during a step test on 3 patients (2 with tuberculosis and emphysema and one with a destroyed left lung).

Arterial oxygen desaturation during exercise has been recorded by an oximeter in patients with congenital heart disease (4, 12, 13).

In the present series, 20 of 24 "normal" subjects (*Groups I*, *II*, and *III*) showed arterial oxygen desaturation during a step test. In 5 of these the fall of arterial oxygen saturation was between 3.0 and 3.8 saturation per cent. Thus a slight fall in arterial oxygen saturation in normal subjects during a step test is usual but a fall greater than 3.8 per cent may be considered abnormal.

In *Group IV* the 4 subjects who showed an abnormal fall in arterial oxygen saturation during exercise all had severe pulmonary disease with either a ventilatory defect or an alveolar-capillary block.

In the patients with emphysema (*Group V*) one could not predict either from clinical disability or from the maximal breathing capacity whether there would be a fall of arterial oxygen saturation during the one-minute step test.

* * *

In the above discussion the oximeter tests have been analyzed individually. When considered as a group their value was increased. Normal subjects had a 90 per cent desaturation time of less than 300 seconds, the hyperventilation index was more than 30 per cent, and on exercise the arterial oxygen saturation did not fall more than 3.8 saturation per cent. Patients with an alveolar-capillary diffusion defect had a normal 90 per cent desaturation time, the hyperventilation index was less than 40 per cent, and on exercise there was a significant fall in arterial oxygen saturation. Subjects with emphysema had a 90 per cent desaturation time of more than 300 seconds (often more than 400 seconds), the hyperventilation index was usually less than 30 per cent, and there might or might not be arterial oxygen desaturation on exercise. Patients with pulmonary disease other than those mentioned above, such as bronchiectasis and simple ventilatory defects, had a 90 per cent desaturation time which was normal. The hyperventilation index was usually less than 50 per cent, and, depending on the severity of the ventilation abnormality, there might or might not be a fall of arterial oxygen saturation during exercise.

Oximeter tests were most useful when combined with simple tests of ventilatory function. Under such circumstances a diffusion defect was indicated by normal or near normal ventilatory function but abnormal arterial oxygen desaturation on exercise; the presence of emphysema was confirmed by a prolonged 90 per cent desaturation time; and the severity of a ventilation defect might be judged in the individual by the effect of exertion on the arterial oxygen saturation.

SUMMARY

A direct-writing, ear oximeter has been used to record changes in arterial oxygen saturation in 65 subjects (34 with normal lungs and 31 with pulmonary disease) during the breathing of oxygen and during hyperventilation and exercise.

The rise of the arterial oxygen saturation during oxygen breathing was, on the average, slower in patients with emphysema than in the other subjects; but, when expressed as the 90 per cent saturation time, there was considerable overlap between the results in the normal and abnormal subjects. The test was not accurately reproducible. The defects were not corrected when the 90 per cent saturation time was related to oxygen consumption and the volume of oxygen breathed during the test. The 90 per cent desaturation time separated patients with diffuse obstructive emphysema, with or without pulmonary fibrosis, from normal subjects and from patients with pulmonary disease other than emphysema, and the results were reproducible with reasonable accuracy. The prolongation of the fall of the arterial oxygen saturation when patients the emphysema were changed from breathing oxygen to breathing air was probably due to inefficient gas mixing. The 90 per cent desaturation time was a useful rapid test for the presence of diffuse obstructive emphysema.

During hyperventilation all subjects showed a rise in arterial oxygen saturation.

Most subjects showed a fall in arterial oxygen saturation during a one-minute 30-step exercise test. For normal persons this was not greater than 3.8 saturation per cent but, in patients with alveolar-capillary block, severe ventilation defects, and moderate to severe emphysema, the fall was considerable.

These oximeter tests were simple to perform, gave useful information, and caused the patient little inconvenience. Their value was increased when combined with simple tests of ventilatory function.

SUMARIO

Sencillas Pruebas de la Función Respiratoria con un Audi-Oxímetro de Escritura Directa

Un audi-oxímetro de escritura directa fué usado para registrar las alteraciones en la saturación arterial con oxígeno en 65 sujetos (34 de pulmones normales y 31 con afección pulmonar) durante la respiración de oxígeno y durante la hiperventilación y el ejercicio.

El aumento de la saturación arterial con oxígeno durante la respiración de oxígeno fué, en conjunto, más lento en los enfisematosos que en los demás sujetos, pero cuando se expresa en tiempo de 90 por ciento de saturación, existe considerable sobreposición entre los resultados en los sujetos normales y los anormales. La prueba no resultó reproducible con exactitud. Los defectos no se rectificaron al relacionarse el tiempo de 90 por ciento de satu-

ración con el consumo de oxígeno y el volumen de oxígeno respirado durante la prueba. El tiempo de 90 por ciento de desaturación separó a los enfermos que tenían enfisema oclusivo difuso, con o sin fibrosis pulmonar, de los sujetos normales y de los que tenían una afección pulmonar distinta de enfisema, y era reproducible con bastante exactitud. La prolongación de la baja de la saturación arterial con oxígeno cuando se cambiaba a los enfisematosos de la respiración de oxígeno a la de aire se debió probablemente a inadecuada mezcla de bases. El tiempo de 90 por ciento de desaturación resultó ser una prueba rápida y útil para descubrir la existencia de enfisema oclusivo difuso.

Durante la hiperventilación, todos los sujetos mostraron elevación de la saturación arterial con oxígeno.

La mayor parte de los sujetos mostró baja de la saturación arterial con oxígeno durante una prueba de 30 pasos de ejercicio en un minuto. En los sujetos normales esto no excedió de 3.8 por ciento de saturación, pero en los que tenían bloqueo alvéolo-capilar, graves deficiencias de la ventilación y enfisema que variaba de moderado a grave, la baja fué considerable.

Estas pruebas oximétricas resultaron sencillas en su ejecución, aportaron datos útiles y ocasionaron poca molestia a los enfermos. Su valor aumentó cuando se combinaban con sencillas pruebas de la función ventiladora.

RESUME

Tests simples de la fonction respiratoire à l'aide d'un "oxymètre-oreille" à inscription directe

Un "oxymètre-oreille" à inscription automatique a été utilisé pour enregistrer les modifications de la saturation artérielle en oxygène de 65 sujets (34 ayant des poumons normaux et 31 atteints de lésions pulmonaires) pendant l'inhalation d'oxygène et au cours de l'hyperventilation et de l'effort.

L'élévation de la saturation du sang artériel en oxygène durant l'inhalation d'oxygène était en moyenne plus lente chez les patients atteints d'emphysème que chez les autres sujets; mais, quand elle était exprimée comme temps de saturation à 90%, il y avait un chevauchement considérable entre les résultats obtenus chez les sujets normaux et anormaux. Ce test n'a pu être reproduit avec exactitude. Les erreurs ne pouvaient être corrigées quand le temps de saturation à 90% était rapporté à la consommation d'oxygène et au volume de l'oxygène inhalé durant le test. Le temps de désaturation à 90% séparait les malades atteints d'emphysème obstructif diffus, avec ou sans fibrose pulmonaire, des sujets normaux et des sujets atteints d'affections pulmonaires autres que l'emphysème et pouvait être reproduit avec une exactitude suffisante. La prolongation de la chûte de la saturation en oxygène du sang artériel quand les malades atteints d'emphysème passaient de l'inhalation d'oxygène à la respiration dans l'air ambiant était probablement due à une déficience du mélange gazeux. Le temps de désaturation à 90% constituait un test rapide utile pour déceler l'emphysème obstructif diffus.

Durant l'hyperventilation tous les sujets ont présenté une élévation de la saturation en oxygène du sang artériel.

La plupart des sujets présentaient une chûte de la saturation en oxygène du sang artériel durant le test d'exercice de la montée de 30 marches en une minute. Chez les individus normaux, ceci ne dépassait pas 3,8 saturation pour cent, mais, chez les malades présentant un bloc alvéolo-capillaire, des troubles sévères de la ventilation et un emphysème de degré variant entre modéré et sévère, la chûte était considérable.

Ces "oxymètres-tests" étaient simples à effectuer, donnaient des informations utiles et ne présentaient guère de difficultés pour le malade. Leur valeur était accrue quand ils étaient associés au simple test de la fonction de ventilation.

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BERYLLIUM GRANULOMATOSIS OF THE LUNGS[1]

A Four-Year Follow-Up of Two Patients

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INTRODUCTION

In the past ten years there has been increasing awareness of the industrial hazards of beryllium exposure. Numerous case reports and several reviews have appeared in the literature (1, 2). Among these reports there have been isolated objective studies of pulmonary function and both clinical and functional observations related to the use of steroid therapy (3, 4). Yet, it has not been possible to find long-term follow-up studies of the pulmonary function studies in any such patient.

Over the past four years the present writers have had the opportunity of observing 2 patients with granulomatous disease of the lungs due to beryllium poisoning. During this period the pulmonary function of these patients has been studied on repeated occasions. The purpose of this paper is to report clinical and functional observations of this disease in these 2 contrasting cases.

OBSERVATIONS

Case Reports

Case 1. A white male, aged 21 years, was exposed to beryllium phosphors for a two month period in 1946 while working in a fluorescent bulb factory. He had no symptoms except those related to a psychiatric disorder for which he was admitted to the Veterans Administration Hospital, Northport, New York, in 1947. A diagnosis was made of schizophrenia (paranoid type), and shock therapy was given. Chest roentgenograms at that time were normal.

The patient's first admission to the Veterans Administration Hospital, Bronx, New York, was in September, 1951, with complaints of dyspnea, frequency of urination, and urethral discharge. There was a history of excessive milk ingestion of two to three quarts daily for five years. Physical examination revealed no abnormalities except axillary lymphadenopathy and an enlarged spleen. A chest roentgenogram revealed extensive infiltrations of both lungs (figure 1*A*). Significant laboratory findings were: a blood urea nitrogen of 28 mg. per 100 ml.; total serum proteins, 7.0 gm., with 4.9 gm. of albumin and 2.1 gm. of globulin; blood calcium 13.0 mg. per 100 ml.; phosphorus 3.2 mg. per 100 ml.; and alkaline phosphatase, 2.4 Shinawara units. Intravenous and retrograde pyelograms revealed right renal calculi. Repeated sputum smears and cultures were negative for tubercle bacilli. A tuberculin skin reaction was positive only to the full "second strength" of PPD.

A kidney biopsy was taken during operation for removal of renal calculi. This tissue as well as that from a lymph node biopsy revealed tuberculoid, noncaseating granulomata compatible with sarcoidosis or berylliosis. A biopsy of the site of a Kveim test yielded normal tissue. From 1952 to 1955 the patient was followed in the chest clinic and the cardiopulmonary laboratory. During this period he gradually developed increasing exertional dyspnea, clubbing of the fingers, and cyanosis.

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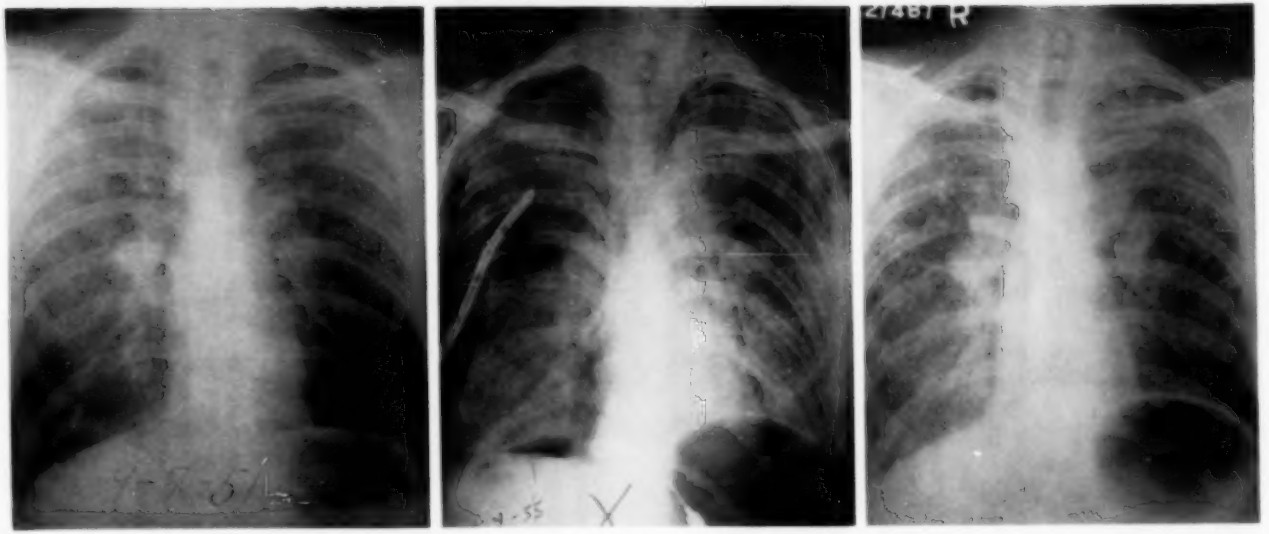


Case 1

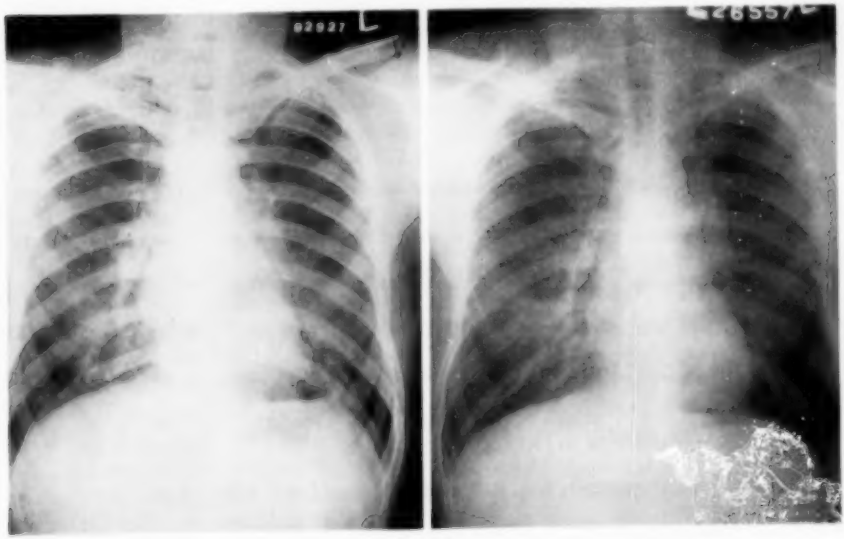
FIG. 1*A* (Left). Chest roentgenogram obtained on first admission in September, 1951.

FIG. 1*B* (Center). Chest roentgenogram obtained on second admission in April, 1955, with spontaneous pneumothorax and mediastinal emphysema.

FIG. 1*C* (Right). Roentgenogram obtained after re-expansion of the lung. Note bullae in right apex.

He was readmitted to the hospital in April, 1955, with a spontaneous right pneumothorax. Tube drainage with high negative pressure was required for re-expansion of the lung. He had a stormy course complicated by the development of severe subcutaneous emphysema. Multiple skin incisions over the chest and neck were required to relieve pressure on the air passages. Two weeks later a left spontaneous pneumothorax and mediastinal emphysema developed (figure 1*B*). Re-expansion of the lungs occurred slowly, over a two-month period, with considerable pleural reaction. Bullae were noted at the apex of the right lung after re-expansion (figure 1*C*). Urinary findings and blood chemistries were normal except for reversal of the serum albumin-globulin ratio (3.3/4.7). The electrocardiogram was normal and there was no cardiac enlargement. At the time of discharge in August, 1955, the patient noted dyspnea with light activity but was able to return to light, sedentary work.

Case 2. A 25-year-old white male was exposed to beryllium in 1943 for a period of six months while working in a room in which defective fluorescent bulbs were destroyed. He reported mild exertional dyspnea and chronic cough beginning in 1944, but the first roentgenographic evidence of extensive bilateral lung infiltration was in 1949. He was admitted to the Veterans Administration Hospital, Bronx, New York, for the first time in May, 1951, for an eye operation and was transferred to the chest service for evaluation of his lung disease. A roentgenogram revealed a fine, reticular infiltrate in both lung fields and hilar nodal enlargement (figure 2*A*). The only physical abnormality was mild clubbing of the fingers. The laboratory findings were all within normal limits. The skin reaction to tuberculin was negative. A liver biopsy yielded normal tissue. Examination of a specimen obtained by lung biopsy gave findings compatible with beryllium granuloma, and the specimen contained traces of beryllium on chemical analysis. Because of the widespread use of steroid therapy in this and related diseases at the time, it was decided to administer cortisone. The patient received 100 mg. daily for two months after which time the dosage was gradually lowered and was discontinued one week later. No subjective improvement was noted on



Case 2

FIG. 2*A* (Left). Chest roentgenogram obtained on first admission in September, 1951.
FIG. 2*B* (Right). Chest roentgenogram showing no change in four years.

TABLE 1

RESULTS OF SERIAL STUDIES ON TWO PATIENTS OVER A FOUR-YEAR PERIOD

	Case 1			Case 2					
	10/5/51	6/4/54	8/1/55	6/11/51	9/21/51	11/15/51	1/8/52	7/26/53	8/9/55
Ventilatory Studies									
Vital capacity (per cent of predicted)	85.00	52.00	49.00	67.00	60.00		67.00	73.00	69.00
Residual volume (per cent of predicted)	76.00	95.00	92.00	91.00	75.00		81.00	96.00	103.00
Total capacity (per cent of predicted)	85.00	62.00	58.00	74.00	67.00		69.00	79.00	77.00
Residual volume/Total capacity									
Total capacity (per cent)	17.70	30.50	31.90	24.30	23.00		23.20	24.10	26.50
Alveolar nitrogen (per cent)*	3.13	2.62	2.48	2.52	2.50		1.35	2.43	1.32
Maximal breathing capacity (per cent of predicted)	103.00	98.00	126.00	93.00	102.00		90.00	88.00	90.00
Ventilation at rest (L/min./sq. m.)	5.46	7.40	6.87	5.75	5.80		6.30	4.85	5.11
Ventilation with exercise (L/min./sq. m.)	12.00	19.10	19.30	14.00	11.70		12.40	11.80	11.20
O_2 consumption at rest (cc./min./sq. m.)	157.00	157.00	157.00	141.00	128.00		139.00	139.00	129.00
O_2 consumption with exercise (cc./min./sq. m.)	473.00	437.00	379.00	502.00	421.00		405.00	397.00	345.00
Gas Exchange at Rest —Room Air									
Arterial O_2 saturation (per cent)	99.50	83.70	80.30	94.60	95.30	93.00	92.50	93.70	95.30
Arterial CO_2 content (vol. per 100 ml.)	43.70	45.40	43.60	46.00	46.50	46.30	43.40	41.80	46.40
Arterial P_{CO_2} (mm. Hg.)	37.00	34.00	32.00	46.00	43.00	42.60	40.00	40.00	42.00
Alveolar P_{O_2} (mm. Hg.)	106.00	108.00	110.00	93.00	101.00		94.00	102.00	102.00
Arterial P_{O_2} (mm. Hg.)	96.00	60.00	52.00	86.00	93.00	92.00	86.00	88.00	96.00
A-A gradient	10.00	48.00	58.00	7.00	8.00		8.00	14.00	6.00
Dead space (per cent tidal air)	35.00	44.00	40.00	48.00	43.00		47.00	34.00	40.00
pH	7.41	7.46	7.43	7.35	7.36	7.38	7.35	7.41	7.37
At Rest—Low Oxygen									
Arterial O_2 saturation (per cent)	72.50	72.50	50.50	82.50	85.50		79.20	83.70	80.80
Arterial CO_2 content (vol. per 100 ml.)	42.80	43.30	42.60	44.70	46.60		42.40	41.90	45.60

TABLE 1 (*Concluded*)

	Case 1			Case 2					
	10/5/51	6/4/54	8/1/55	6/11/51	9/21/51	11/15/51	1/8/52	7/26/53	8/9/55
Arterial P_{CO_2} (mm. Hg.)	37.00	29.00	30.00	46.00	47.00		33.00	40.00	39.00
Alveolar P_{O_2} (mm. Hg.)	39.00	79.00	69.00	52.00	54.00		65.00	65.00	61.00
Arterial P_{O_2} (mm. Hg.)	37.00	33.00	26.00	47.00	47.00		38.00	46.00	45.00
A-A gradient	2.00	46.00	43.00	5.00	7.00		27.00	19.00	16.00
Dead space (per cent tidal air)	47.00	45.00	41.00	53.00	50.00		41.00	37.00	39.00
pH	7.40	7.50	7.49	7.34	7.43		7.35	7.41	7.42
With Exercise—Room Air									
O_2 saturation	90.70	73.30	57.60	81.80	82.60	77.30	74.50	87.60	86.40
CO_2 content	43.00	42.90	40.30	42.40	45.30	47.40	43.10	41.60	43.00

* After breathing 100 per cent O_2 for seven minutes.

therapy. The patient was discharged from the hospital and periodically studied in the laboratory. There has been no change in symptoms or roentgenographic findings (figure 2*B*). The patient is able to carry on his occupation as a golf caddie without difficulty.

RESULTS OF PULMONARY FUNCTION STUDIES

Ventilation and gas exchange studies were performed, using standard procedures described elsewhere (5). Serial studies were done on each patient over a four-year period and the results are tabulated in table 1.

Case 1. The first study, performed five years after exposure to beryllium, revealed a slight decrease in the lung volumes, slight hyperventilation at rest and during standard exercise, and slight arterial oxygen unsaturation during exercise.

Repeat studies thirty-two months later revealed a marked worsening of ventilatory and alveolar-respiratory function. The vital capacity and total lung capacity were 52 per cent and 62 per cent of normal, respectively, and the minute ventilation was twice the normal value both at rest and during exercise. The maximal breathing capacity remained normal. Gas exchange was markedly impaired, resulting in an arterial oxygen saturation of 83.7 per cent at rest and 73.3 per cent during standard exercise. The low blood carbon dioxide tension (P_{CO_2}) reflected the marked degree of hyperventilation. Analysis of blood gas studies, performed while breathing room air and a low oxygen mixture, demonstrated large and significant gradients between alveolar and arterial oxygen tensions (P_{O_2}). The complete picture was that of the "alveolar-capillary block" syndrome (6).

One year later, several months after the bilateral pneumothorax, pulmonary function studies revealed further deterioration as manifested by an arterial oxygen

saturation of 80.3 per cent at rest and 57.6 per cent during exercise. The other abnormalities were similar to those noted in the preceding study.

Case 2. The pulmonary function of the second patient was studied on six different occasions. The first study was performed eight years after exposure to beryllium; the second, three weeks after thoracotomy and lung biopsy; the third and fourth while the patient was on cortisone therapy; and the last two as more recent follow-up studies.

There was a moderate reduction of vital and total capacities, mild hyperventilation at rest and during standard exercise, and significant reduction in the arterial oxygen saturation during exercise but not at rest. In comparative studies the results were remarkably constant. Blood gas studies revealed the development, after cortisone therapy, of a significant oxygen gradient between alveolus and capillary while breathing a low oxygen mixture, indicating a mild alveolar-capillary block pattern.

DISCUSSION

The criteria for the diagnosis of beryllium granulomatosis have been described by Hardy (1) and include: beryllium exposure and/or its presence in the tissues plus a granulomatous pathologic reaction. Although no beryllium was found in the tissues of the first patient, the history of exposure is well documented and the biopsy findings are compatible. The negative Kveim reaction and the clinical course in a white male are further evidence against the diagnosis of sarcoidosis. In the second patient, all of the criteria for the diagnosis of beryllium granulomatosis were present.

These 2 cases resemble many of those reported in that there is a latent period of several years between exposure to beryllium and the appearance of symptoms, clinical findings, or roentgenographic changes. Once the disease is clinically evident, the severity and rapidity of progression may be quite dissimilar as the contrasting courses of these 2 patients demonstrate. It is obvious that the pathologic processes and the functional pattern are qualitatively similar but differ markedly in degree. The fact that many organ systems may be involved is apparent in *Case 1*, in which lung, kidney, and lymph node were involved in the granulomatous process.

The pulmonary function studies confirm previous reports (3, 4) indicating that, in the more advanced stages of the disease, interstitial fibrosis may lead to alveolar-capillary block. In the second patient there was a slow development of this pattern over a period of four to five years, at the end of which time there was only moderate clinical and functional impairment. The remarkable stability of function in serial studies correlates well with the unchanging symptomatology and the lack of progression of the roentgenographic findings.

In the same period of time, the first patient exhibited a rapid deterioration of pulmonary function and the development of bullous emphysema as a complication of organizing fibrosis of the lungs. The bilateral spontaneous pneumothoraces undoubtedly were the result of "bleb formation" and resembled some of the

features of advanced Shavers' disease (7), which frequently terminates with tension pneumothorax.

Steroid therapy has been advocated for this disease, and earlier reports (3) suggested favorable results, but apparently the enthusiasm has waned more recently. The second patient was treated with cortisone, and no subjective or objective change was noted. It is unlikely that the relatively benign course of this patient can be attributed to the effect of the hormone. The first patient was not treated with steroids because of the fear of precipitating an acute psychotic episode.

SUMMARY

Two contrasting cases of beryllium granulomatous disease of the lung have been presented. Long-term clinical observation and serial pulmonary function tests confirm previous clinical observations, suggesting a variable course of this disease.

SUMARIO

Granulomatosis Pulmonar por Berilio. Cuatro Años de Observación de Dos Enfermos

Preséntanse en contraste 2 casos de granulomatosis pulmonar producida por berilio. La observación clínica a largo plazo y pruebas seriadas de la función pulmonar confirman anteriores observaciones clínicas, que indican la evolucion variable de esta dolencia.

RESUME

Granulomatose au béryllium pulmonaire: Observation de deux malades pendant une période de 4 années

Deux cas différents de lésions granulomateuses du poumon provoquées par le béryllium ont été présentées. L'observation clinique prolongée et des tests en série de la fonction pulmonaire confirment les observations cliniques antérieures en suggérant que cette maladie a un cours variable.

Acknowledgment

The writers wish to thank Mrs. Blanche Devlin of the cardiopulmonary laboratory for the technical assistance which made this paper possible. The illustrations utilized in this paper were prepared under the direction of Mr. David Lubin, Medical Illustration Department, VA Hospital, Bronx, New York.

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ANTITUBERCULOUS IMMUNITY INDUCED IN MICE BY VIRULENT PRIMARY INFECTION[1]

Its Inhibition by Chemoprophylaxis

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INTRODUCTION

The increased resistance to experimental tuberculosis brought about in mice by vaccination with BCG can be measured in terms of two different criteria: the numbers of living virulent bacilli present in the organs of animals sacrificed at various intervals of time after a small challenge infection, and the survival time of animals infected with a large dose of virulent culture. These two criteria have been used in the present study to demonstrate that an increase in resistance can also result from nonprogressive primary infections caused by small numbers of virulent bacilli. It will be shown furthermore that treatment of the animals with isoniazid at the time of the primary infection does interfere with the development of the immune state.

MATERIALS AND METHODS

Two virulent strains of bovine tubercle bacilli have been used in the present study. One is a eugonic strain (MV) which produces large, dense, wrinkled colonies on oleic-acid–albumin agar (1). The other is a dysgonic strain (Dg2) which produces on the same medium colonies that remain flat, relatively small, and transparent even after prolonged incubation (2). Both strains are highly virulent for mice. Intravenous injection into young albino mice of 0.01 ml. of culture in Tween®-albumin medium of either strain MV or Dg2 uniformly results in an extensive pulmonary disease with fatal outcome.

All experiments were carried out in albino mice, four to six weeks old, of the so-called Rockefeller Institute Swiss breed. The animals were maintained in metal cages and fed, ad libitum, pellets and water or bread and milk. They were infected by the injection of appropriate dilutions of culture (in a final volume of 0.2 ml. of 0.1 per cent bovine albumin) into one of the caudal veins. The numbers of bacilli in the organs were determined by techniques described elsewhere (3).

OBSERVATIONS

Fate of Bacilli of Superinfection in Mice Having Undergone a Primary Infection

The bacteriologic analysis of primary infection and of superinfection would be greatly facilitated if it were possible to follow in the same animal the separate fate of the bacteria involved in the two infectious processes. Unfortunately, this type of study is often difficult, as ordinary cultural or immunologic techniques rarely permit convincing differentiation of one culture of virulent mammalian mycobacteria from another. An approach to this problem was made by Boquet and Laporte who showed that, in guinea pigs and rabbits, primary infection with

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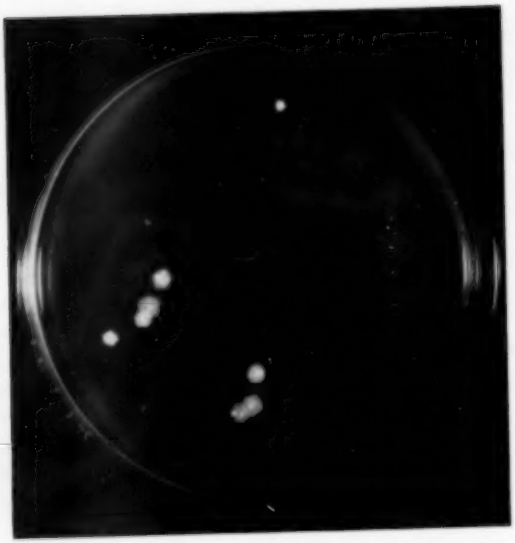

FIG. 1. Eugonic colonies of bovine tubercle bacilli of the MV strain, recovered directly from spleen of mouse sacrificed sixteen weeks after intravenous injection of 0.2×10^{-4} ml. of culture.

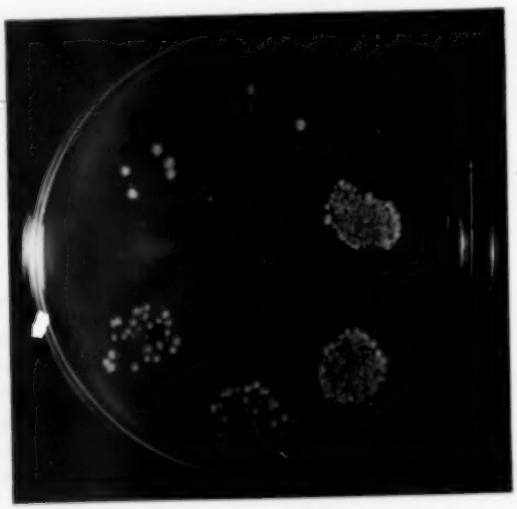

FIG. 2. Dysgonic colonies of bovine tubercle bacilli of the Dg2 strain, recovered directly from spleen of mouse sacrificed eight weeks after intravenous injection of 0.2×10^{-4} ml. of culture.

a dysgonic strain of tubercle bacilli retarded the spread of eugonic bacilli of superinfection (4). In the experiment now to be described, the eugonic bovine strain MV was used for primary infection of mice, and the dysgonic strain Dg2 for superinfection. The contrast in colonial morphology exhibited by these two strains on oleic-acid–albumin agar readily permitted their identification in cultures of mouse organs harboring a mixed infection (figures 1, 2, 3).

Two groups of mice received by the intravenous route either 0.2×10^{-6} or 0.2×10^{-7} ml. of a ten-day-old culture in Tween-albumin medium of the eugonic strain MV. Colonial enumeration on albumin agar revealed that these infective doses contained 200 or 20 viable units, respectively. At various intervals of time after primary infection, groups of 4 animals were superinfected by the intravenous injection of 0.2×10^{-6} ml. of the dysgonic culture Dg2. Control animals (without primary infection) were also infected with Dg2 at the same time. The Dg2 infective dose consisted in all cases of 0.2×10^{-6} ml. of culture in Tween-albumin medium and contained approximately 300 viable units.

Four animals of each group were sacrificed at various intervals of time after the superinfection, and the numbers of viable bacilli in their spleens and lungs were determined by plating appropriate dilutions of these organs on oleic-acid–albumin agar. The eugonic and dysgonic colonies were counted after three weeks' incubation at 37°C. (table 1). The appearance of the two types of colonies recovered from the organs are illustrated in figures 1, 2, and 3.

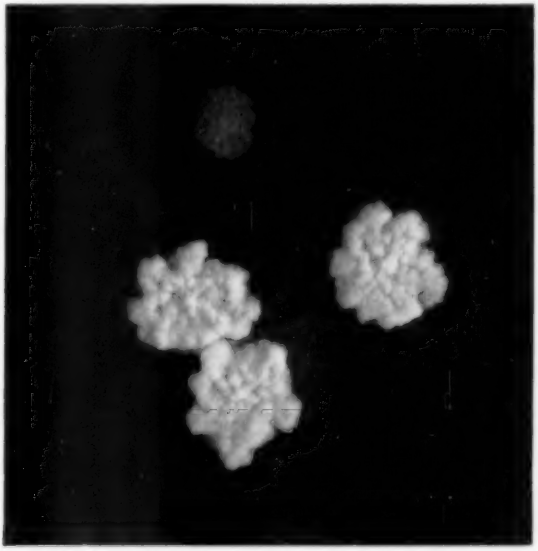

FIG. 3. One dysgonic (Dg2) and 3 eugonic (MV) colonies, recovered directly from lung of mouse sacrificed sixteen weeks after intravenous injection of 0.2×10^{-6} ml. of MV culture and eight weeks after superinfection with 0.2×10^{-6} ml. of Dg2 culture.

In all cases, the organs were ground in 5 ml. of 2 per cent bovine albumin, and drops of appropriate dilutions were deposited on the surface of oleic-acid–albumin agar. The colonies were photographed after nineteen days' incubation at 37° C.

The results presented in part *A* of table 1 illustrate the course of infection caused by injecting small inocula of virulent bacilli of the MV strain. These results are very similar to those reported in an earlier communication from this laboratory (3). Large numbers of colonies of the MV organisms used for primary infection were recovered from the spleens of the animals inoculated with the larger dose (200 viable units) and sacrificed three weeks after infection. The numbers were smaller for the lungs. With this infective dose the numbers of colonies decreased after the third week, but increased again later, particularly in the lungs (*see* animals sacrificed sixteen weeks after infection). Far fewer colonies were recovered from the spleens and the lungs of mice which had received a very small primary infection (20 viable units). Nevertheless, it should be noted that positive cultures could still be obtained from these animals sixteen weeks after infection.

It is apparent that the culture used for superinfection (Dg2) was highly virulent, as shown by the large numbers of colonies recovered from the spleens and lungs five weeks and eight weeks after injection of 300 viable units (part *B* of

TABLE 1

EFFECT OF PRIMARY INFECTION WITH TUBERCLE BACILLI (MV) ON FATE OF BACILLI OF SUPERINFECTION (Dg2)

A. Course of Primary Infection (MV) Without Superinfection

Viable Units of MV Injected		Numbers of MV Colonies* Recovered at Indicated Times After Primary Infection		
		3 weeks	8 weeks	16 weeks
200	Spleen	56,000	800	2,100†
200	Lungs	1,000	0	12,000†
20	Spleen	800	200	200
20	Lungs	0	0	200

B. Primary Infection (MV) Followed by Superinfection (Dg2)

Viable Units of MV Injected		Numbers of Colonies* Recovered from Mice Superinfected at Indicated Times After Primary Infection			
		3 weeks‡		8 weeks§	
		MV	Dg2	MV	Dg2
200	Spleen	2,000	8,000	200	16,000
200	Lungs	200	800	600	600
20	Spleen	1,000	26,600	200	32,800
20	Lungs	0	16,400	600	54,800
0 (controls)	Spleen	—	94,000	—	145,600
0 (controls)	Lungs	—	178,000	—	202,000

* Per whole organ.

† The individual counts on this group of 4 mice were as follows:

Spleen	4,600	600	?	1,600
Lungs	30,000	18,000	4,000	0

‡ Sacrificed five weeks after superinfection.

§ Sacrificed eight weeks after superinfection.

table 1). Clearly also, the course of the superinfection was much less severe in mice which had been subjected to primary infection with the MV strain than in the controls. Evidence of immunity could be detected even in animals which had received only 20 viable units of the MV culture. It is worth noting that the immune process did not succeed in eliminating the few organisms resulting from the primary infection even though it was so highly effective in retarding proliferation of the bacteria of superinfection.

Effect of Chemoprophylaxis During Primary Infection on the Fate of the Bacteria of Superinfection

As may be seen in table 1, the numbers of MV colonies recovered from the spleen and lungs three weeks after primary infection were far greater than the numbers of viable units injected per mouse (20 and 200 viable units). It is furthermore known on the basis of many other experiments that positive cultures would have been obtained at that time from practically all of the organs of the infected animals. There is no doubt, therefore, that the tubercle bacilli of the MV strain had multiplied *in vivo* extensively, even in the animals which had received the smallest infective dose (20 viable units). This fact made it of interest to determine what effect treatment with antibacterial drugs might have on the immunizing effect of primary infection.

Mice received by the intravenous route 0.2×10^{-8} ml. of bovine tubercle bacilli of the MV strain. Some of the animals were treated with isoniazid for one week before primary infection and for four weeks thereafter. The drug was added to the drinking water (given ad libitum) in amounts sufficient to provide for an approximate daily intake of 5 mg. Two weeks after discontinuance of the drug, i.e., six weeks after primary infection, some of the animals were superinfected with 0.2×10^{-4} ml. of H37Rv culture. Various types of control groups were included in the experiment, as indicated in table 2. The animals were sacrificed two weeks after superinfection with H37Rv, and the total numbers of bacteria in their spleens and lungs were determined as usual (table 2).

TABLE 2

EFFECT OF CHEMOPROPHYLAXIS DURING PRIMARY INFECTION ON THE FATE OF TUBERCLE BACILLI OF SUPERINFECTION

Primary Infection Bovine Tubercle Bacilli MV (ml. intravenously)	Prophylactic* Isoniazid (ml. daily)	Superinfection Human Tubercle Bacilli H37Rv (ml. intravenously)	Numbers of Colonies Recovered from Spleen†	
			6 weeks after primary infection	2 weeks after superinfection
0.2×10^{-8}	0	0	18,000 (MV)	2,800 (MV)
0.2×10^{-8}	5	0	0	0
0.2×10^{-8}	0	0.2×10^{-4}	18,000 (MV + H37Rv)	21,000 (MV + H37Rv)
0.2×10^{-8}	5	0.2×10^{-4}	0	980,000 (MV + H37Rv)
0 (controls)	0	0.2×10^{-4}	—	1,030,000 (MV + H37Rv)

* One week before and four weeks after primary infection.

† For whole organ; average of 4 animals.

As may be seen from the results presented in table 2, primary infection with 0.2×10^{-6} ml. of the MV culture of bovine tubercle bacilli markedly reduced the severity of the infectious process in mice superinfected with 0.2×10^{-4} ml. of the human strain H37Rv six weeks later. As was to be expected, chemoprophylaxis with isoniazid prevented the multiplication of the bacilli of primary infection. It is obvious, furthermore, that the numbers of bacilli of superinfection in animals which had received the drug were of the same order as those found in animals which had not been subjected to primary infection. In other words, isoniazid chemoprophylaxis had interfered with the development of the immune response.

Effect of Chemoprophylaxis During Primary Infection on the Survival Time of Mice After Superinfection

The following experiment was designed to test the effect of primary infection, with and without chemoprophylaxis, on the survival time following superinfection with large doses of virulent bacilli.

Mice received, by the intraperitoneal route, a primary infection consisting of either 0.1 ml. or 10^{-6} ml. of the virulent MV culture. Some of the animals were placed on isoniazid chemoprophylaxis for one week before and eight weeks after primary infection. The drug was administered in the drinking water (given ad libitum) in concentration sufficient to provide an approximate daily intake of 5 mg. per animal. Appropriate control groups were included, as indicated in table 3. All animals were superinfected with 0.1 ml. of culture MV ten weeks after primary infection, i.e., two weeks after discontinuance of isoniazid.

The results presented in table 3 throw light on the complex relationships that exist between size of the primary infective dose, isoniazid prophylaxis, and immune response. It may be seen that primary infection with a small dose of virulent culture (10^{-6} ml.) administered intraperitoneally resulted in a marked increase in survival time of mice superinfected ten weeks later by the intravenous injection of a large dose of culture (0.1 ml.). In this case as in the pre-

TABLE 3

EFFECT OF CHEMOPROPHYLAXIS DURING PRIMARY INFECTION WITH TUBERCLE BACILLI ON THE SURVIVAL OF MICE AFTER SUPERINFECTION

Primary Infection Bovine Tubercle Bacilli MV (ml. intraperitoneally)	Prophylactic Isoniazid (mg. daily)	Cumulative Numbers of Deaths at Indicated Times After Superinfection*				
		4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
10^{-1}†	5	2	3	4	8	8
10^{-6}	5	3	8	15	16	17
10^{-6}	0	2	3	5	7	9
0	5	1	6	15	16	19
0	0	2	7	14	15	18

* Of 20 mice infected intravenously with 0.1 ml. virulent culture MV. Ten weeks after primary infection; isoniazid discontinued two weeks before challenge superinfection.

† Animals infected with this amount of culture, without isoniazid treatment, died in twelve to thirty days after infection.

ceding experiment, isoniazid chemoprophylaxis prevented the development of the immune response. In contrast, chemoprophylaxis had no inhibitory effect on the development of immunity in mice which had received a very large primary infective dose (0.1 ml. intraperitoneally). The reason for this apparent conflict in the results obtained with large and small infective doses will be considered subsequently in the discussion.

DISCUSSION

The intravenous injection into mice of very small doses of virulent tubercle bacilli, even those containing but a few viable units, results almost uniformly in an infectious process which remains active for a few weeks (3). In general, however, bacillary multiplication eventually stops or at least becomes extremely slow, and the animal survives. The ultimate result is then usually the establishment of a dormant infection characterized by the fact that virulent bacilli persist in small numbers in the various organs. (For a discussion of dormant infections in mouse tuberculosis *see* reference (5).) This state of dormant infection—also referred to as premunition—is associated with a definite level of antituberculous immunity which lasts for many months at least. The present paper deals with the relative immunity elicited in mice by a primary virulent infection that has thus run an abortive course. The results show that this immunity manifests itself in increased survival time following a challenge superinfection, and in smaller numbers of superinfection bacilli in the organs of the challenged animals.

An attempt has been made to follow simultaneously the separate fate of the bacilli of primary infection and of superinfection in a given mouse. This has been achieved by establishing the primary infection with a eugonic strain and the superinfection with a dysgonic strain of virulent bacilli. This method takes advantage of the fact that these two strains produce colonies with a strikingly different morphology on oleic-acid–albumin agar (2). The results obtained by this technique make clear that when the eugonic organisms of the primary infection become established in the mouse—even in very small numbers—the multiplication of the dysgonic organisms of superinfection is very much reduced. Yet the organisms of primary infection continue to survive in the organs of the superinfected animals.

Under normal circumstances, injection of amounts as small as 10^{-7} ml. or 10^{-6} ml. of culture, corresponding to 20 or 200 viable units of tubercle bacilli, was sufficient to elicit a protective effect against superinfection. With these minute primary infective doses, however, the immune response did not occur if the animals received isoniazid at the time of the primary infection. In other words, it appeared that the immune state developed only if the bacilli of primary infection were allowed to multiply *in vivo*.

The effect of primary infection and of chemoprophylaxis on immunity was also studied by using as criterion the survival time of the superinfected animals, instead of the fate *in vivo* of the bacilli of superinfection. Mice which had undergone primary infection with small doses of virulent bacilli survived the super-

infection longer than did the controls; but this protective effect was not obtained if the animals had received isoniazid at the same time as the primary infective dose. In contrast, chemoprophylaxis did not prevent the development of immunity if the dose of bacilli used for primary infection was large (0.1 ml. of culture).

The findings described in the present paper are consistent with those of experiments reported elsewhere in which tubercle bacilli of the R1Rv or BCG strains were used as immunizing agents (6, 7). In all cases, injection of a small primary infective dose, whether of virulent bacilli, of R1Rv, or of BCG, resulted in a sufficiently extensive bacterial multiplication *in vivo* to elicit a protective response. But immunity did not develop when proliferation of the bacilli introduced by primary infection or by vaccination was inhibited as a result of treatment with isoniazid. When the primary infective dose (or dose of vaccine) was very large, on the other hand, it provided outright an amount of bacterial protoplasm sufficiently large to produce an effective antigenic stimulus, even though the drug prevented the bacilli of primary infection or of vaccination to multiply *in vivo*.

It is thus apparent that, while chemoprophylaxis does not affect the immune response to the bacilli present in the body, it can interfere indirectly with the development of immunity by inhibiting bacillary multiplication *in vivo*. These findings are relevant to recently published statements according to which treatment with isoniazid does not prevent the development of antituberculous immunity in guinea pigs infected with the H37Rv strain (8) or in mice vaccinated with BCG (9). As pointed out in an editorial in this journal (10), the statements under consideration were based on experiments in which the infective inocula consisted of amounts of H37Rv or BCG cultures so large that the antibacterial effect of isoniazid could play no inhibitory role in the immune response.

The complex relationships recognized in mice between infective dose, chemoprophylaxis, and immunity probably have a bearing on the problems of tuberculous infection in man. As is well known, only very small particles (1–5 micra) can reach pulmonary tissue under normal circumstances; the doses of tubercle bacilli responsible for primary infection in man must, therefore, be relatively small even under conditions of heavy exposure. In consequence, the quantities of antigen (in terms of weight of bacterial protoplasm) introduced by air-borne infections can hardly ever be sufficient to elicit a significant immunologic response. Since the main source of the antigenic stimulus comes from the organisms that multiply *in vivo*, rather than from the bacteria present in the primary infective inoculum, it can be assumed that the immune response would remain inadequate in persons receiving isoniazid at the time primary infection occurred.

SUMMARY

The fate of bacilli of primary infection and of superinfection was studied in mice by using eugonic and dysgonic strains of virulent bovine bacilli as respective inocula for the two infectious processes. These organisms were selected because

they produce colonies on oleic-acid–albumin agar which can be distinguished morphologically even when recovered simultaneously from organs with mixed infections.

It could be shown by this technique that the multiplication of the bacilli of superinfection was much reduced in the organs of animals harboring very small numbers of bacilli of primary infections, even though the immune process did not bring about the elimination of the latter organisms.

The immune state could be elicited by using primary infective inocula containing as few as 20 viable units. It did not develop when the animals were treated with isoniazid at the time of primary infection with small inocula. In contrast, immunity developed even during isoniazid chemotherapy when the primary infective inoculum was very large.

It is concluded from these findings that isoniazid does not affect the antigenic response to the bacteria present in the body, but, nevertheless, interferes indirectly with the development of immunity by inhibiting bacillary multiplication *in vivo*.

The infective dose of tubercle bacilli that reaches pulmonary tissue in man is always relatively small, even under conditions of heavy exposure. It is probable, therefore, that under natural circumstances no significant degree of antituberculous immunity could develop in persons receiving isoniazid chemoprophylaxis at the time when primary infection occurred.

SUMARIO

Inmunidad Antituberculosa Inducida en los Ratones por Infección Primaria Virulenta: Su Inhibición por la Quimioprofilaxis

El destino de los bacilos de la infección primaria y de la superinfección fué estudiado en los ratones, usando cepas eugónicas y disgónicas de bacilos bovinos virulentos como respectivos inóculos para los dos procesos infecciosos. Escogiéronse esos microbios por producir en agar-albúmina-ácido oleico colonias que pueden distinguirse morfológicamente, aun al ser obtenidas simultáneamente de órganos con infecciones mixtas.

Con esta técnica pudo demostrarse que la multiplicación de los bacilos de la superinfección se reducía mucho en los órganos de animales que alojaban cantidades muy pequeñas de bacilos de infecciones primarias, aunque el proceso inmunológico no lograra la eliminación de estos últimos microbios.

Podía incitarse el estado de inmunidad usando inóculos infectivos primarios que no contenían más de 20 unidades viables. No se produjo aquél cuando se trataba a los animales con isoniacida en el momento de la infección primaria con pequeños inóculos. En contraposición, apareció inmunidad aun durante la isoniacidoquimioterapia cuando el inóculo infectivo primario era muy grande.

Dedúcese de estos hallazgos que la isoniacida no afecta la respuesta antigénica a las bacterias presentes en el cuerpo, pero sí obstruye indirectamente la formación de inmunidad por inhibir la multiplicación bacilar *in vitro*.

La dosis infectiva de bacilos tuberculosos que llega al tejido pulmonar en el hombre es siempre relativamente pequeña, aun en condiciones de intensa exposición. Por lo tanto, es probable que, en circunstancias naturales, no se pueda formar inmunidad antituberculosa de mayor alcance en personas que reciban quimioprofilaxis con isoniacida en el momento en que tuvo lugar la infección primaria.

RESUME

Immunité antituberculeuse provoquée chez la souris par une infection primaire virulente: Son inhibition par la chimioprophylaxie

Le sort des bacilles de l'infection primaire et de la surinfection fut étudié chez la souris en utilisant des souches bovines eugoniques et dysgoniques virulentes comme inocula respectifs pour ces deux processus infectieux. Le choix de ces germes a été déterminé en raison de leur aptitude à produire sur gélose à l'acide oléique-albumine des colonies qui peuvent être distinguées morphologiquement, même quand elles sont recouvrées simultanément à partir d'organes affectés d'infections mixtes.

Cette technique a démontré que la multiplication des bacilles de surinfection était fortement réduite dans les organes d'animaux hébergeant des bacilles d'infection primaire peu nombreux, même lorsque le processus d'immunité n'entraînait pas l'élimination de ces derniers.

L'état immun pouvait être provoqué par l'usage d'inocula d'infection primaire ne renfermant que 20 unités viables. Il ne se produisait pas quand les animaux étaient traités par l'isoniazide au moment de l'infection primaire avec inocula réduits. Au contraire, l'immunité se développait au cours de l'isoniazidothérapie quand l'inoculum infectant était important.

Ces résultats ont permis de conclure que l'isoniazide n'affecte pas la réponse antigénique aux bactéries présentes dans l'organisme, mais qu'elle intervient indirectement dans le développement de l'immunité en inhibant la multiplication bacillaire *in vivo*.

Chez l'homme, la dose infectante de bacilles tuberculeux atteignant le tissu pulmonaire est toujours relativement faible, même quand les conditions d'exposition à la contagion sont marquées. Il est donc probable que, dans les circonstances naturelles, il ne peut se produire une immunité antituberculeuse notable chez les sujets soumis à une chimioprophylaxie par l'isoniazide au moment où survient l'infection primaire.

Addendum

Studies on "The effect of isoniazid in experimental corneal tuberculosis," recently published by Robson and Dideock (11), have yielded results consistent with those reported in the present paper. These writers found that, when the development of the corneal lesion is prevented by the administration of isoniazid at the time of infection, subsequent inoculation of the second eye produces a lesion that cannot be differentiated from that developing in a normal animal. In contrast, the lesion does not develop in the second eye in animals not treated at the time of the first infection. With this experimental model as with our own, therefore, prophylactic chemotherapy prevents the establishment of immunity.

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PHAGOCYTOSIS OF M. TUBERCULOSIS AND M. SMEGMATIS STAINED WITH INDICATOR DYES[1, 2]

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INTRODUCTION

Many interesting studies have been conducted, the majority in the past thirty-five years, using indicator dyes as a means of determining the relative hydrogen-ion concentration of the granules, cytoplasm, and the nucleus of both plant and mammalian cells.

In recent years, the internal environment of the cell, especially in regard to the acidity produced, has been of considerable interest. The work of Rous (1), in which he concluded that the acidity of the mammalian macrophage granules may become as low as pH 3.0 or lower when extraneous material such as indicator dyes is stored by the cell in granular form, has been referred to frequently in recent literature (2–4). In the early studies of streptomycin, it was noted that the antimicrobial action of the drug was appreciably reduced by lowering the pH of the environment to below 6.0 (5). Moreover, in 1954, McDermott and Tompsett (4) found that the antituberculous activities of nicotinamide and pyrazinamide vary with the degree of acidity of the environment. Streptomycin is most effective above neutrality in a pH range of 8.0 to 9.0, whereas nicotinamide and pyrazinamide are highly active *in vitro* at pH 5.0 to 5.5 and inactive within the physiologic ranges of pH. Thus, if the pH of the macrophage is lower than pH 6.0, streptomycin might not kill or inhibit the intracellular phagocytized bacteria (6, 7), but its action might be limited to organisms in the extracellular fluids. In contrast, Mackaness (8) has currently shown that pyrazinamide and nicotinamide, which are active *in vitro* only in an acidic environment, are highly active in monocytes *in vitro* when the extracellular environment of the monocytes is maintained at pH 7.0.

Since the *in vitro* work with drugs has created interest in the environmental acidity surrounding phagocytized tubercle bacilli, this study was undertaken to determine the reaction about individual cells and clumps of *M. tuberculosis* and *M. smegmatis* situated within phagocytes. The bacilli were stained with indicator dyes when they were phagocytized by the monocyte and neutrophil of the mouse and guinea pig *in vivo*. In addition, an attempt was made to determine whether there might be a difference in the degree of acidity produced in the phagocytic cells of the mouse and in the cells of the guinea pig, which is generally thought to be more susceptible to the disease. The injection of sulfonphthalein dyes into mice also was reinvestigated.

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[2] This report is based on data submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Bacteriology and Public Health at Michigan State University.

MATERIALS AND METHODS

Animals: All animals were from stock bred at the Michigan Department of Health Laboratories, Lansing, Michigan. Male white mice weighing 27 to 30 gm. and male guinea pigs weighing 250 to 300 gm. were used.

Indicator dyes: The sulfonphthalein dyes used were National Aniline and Chemical Company products. These dyes are very stable chemically and are less affected by proteins and the presence of neutral salts (9) than are a large number of other dyes. Moreover, they are not subject to loss of color on reduction. Dyes used to stain killed organisms were prepared according to the method of Mallmann and Churchill (10), which consists of making 1 per cent solutions of the indicator in 95 per cent alcohol and adding 0.5 per cent concentrated hydrochloric acid. Dyes injected into mice were prepared by placing 100 mg. of the indicator in a sterile mortar and adding the amount of sterile N/20 sodium hydroxide as indicated by Clark (11). The solution was made up to volume, 5 or 10 ml., with 0.9 per cent saline. Since in some experiments 2 per cent solutions were too toxic for the mice, 1 per cent solutions were used and more injections were given. The indicator dyes used are shown in table 1.

Bacterial suspensions: M. smegmatis and *M. tuberculosis* var. *hominis*, strain H37Rv, were obtained from the stock cultures of the Michigan Department of Health Laboratories, Lansing, Michigan. The organisms were grown in Dubos liquid medium for one week at 37°C. The bacilli were killed by flowing steam, and the medium was centrifuged. The

TABLE 1

INDICATOR DYES

Indicator	Full Acid Color	Full Alkaline Color	pH Interval	pK Value
Tetrabromophenol-sulfonphthalein (brom phenol blue)	Yellow	Blue	3.0–4.6	4.1
Tetrabromo-m-cresol-sulfonphthalein (brom cresol green)	Yellow	Blue	3.8–5.4	4.7
Dibromo-o-cresol-sulfonphthalein (brom cresol purple)	Yellow	Purple	5.2–6.8	6.3

TABLE 2

COLOR OF STAINED ORGANISMS IN MACILVAINE'S BUFFER SOLUTIONS

pH of Buffer Solution	Color of Organisms		
	Brom Phenol Blue	Brom Cresol Green	Brom Cresol Purple
2.8	Yellow-green	Yellow	
3.0	Yellow-green	Yellow	
3.4	Blue-green	Yellow	
4.0	Blue-green	Yellow-green	
4.4	Blue	Yellow-green	
4.8	Blue	Green	Yellow
5.2		Blue-green	Yellow
5.6		Blue	Yellow-green
6.0		Blue	Yellow-green
6.4		Blue	Yellow-green
6.6			Blue
6.8			Blue

sedimented organisms were washed with 0.9 per cent sterile saline and re-suspended in a small amount of saline.

Procedure for staining organisms: Two-tenths of a milliliter of packed organisms, suspended in 1 ml. of 0.9 per cent saline, was placed in a 15 ml. centrifuge tube. Four milliliters of the indicator were added, and the tube was inverted several times to mix the organisms with the dye. The dye suspension was stored at least one week, more frequently two weeks, at 22° to 23°C. before use in experiments. The suspension then was centrifuged until the stained bacteria were well packed. The supernatant dye was decanted and the sedimented organisms were washed twice with aqueous buffer solution (pH 7.0). After this treatment, the color of the organisms shifted to the basic side of the indicator.

Color of stained organisms in buffer solutions: To ascertain whether the organisms stained with the sulfonphthalein indicator dyes would change color in the designated pH range, buffer solutions according to MacIlvaine (12) were prepared in a pH range varying from 2.8 to 6.8. After the bacteria had been washed with aqueous buffer solution (pH 7.0) several times, the organisms were placed in the buffer solutions of known pH value, and the color change in the stained bacilli was observed both macroscopically and microscopically. The results are shown in table 2.

Both brom phenol blue and brom cresol purple have a certain disadvantage in that they both manifest "dichromism," which is the quality or condition of presenting one color in reflected and another in transmitted light. Thus, the color of the indicator, depending on the depth of the liquid observed and on the concentration of the indicator, may appear blue in a thin layer of a solution and purple when viewed through a greater depth. This condition, however, did not cause any difficulties in the interpretation of the experimental results, as either the blue or purple color is associated with alkalinity and both change to yellow in the acid range of the indicator.

Procedure for phagocytosis experiments with stained organisms: Mice. White mice were injected intraperitoneally on 2 successive days with 2.0 ml. of 7.2 per cent sodium caseinate (13), which was used as a chemotaxic agent to increase phagocytic cells in the peritoneal exudate. Six hours following the second injection, 0.1 ml. of the packed stained organisms suspended in aqueous buffer solution (pH 7.0) was inoculated into the peritoneal cavity. At intervals of two, twenty-four, forty-eight, seventy-two, and ninety-six hours a small amount of the peritoneal fluid was withdrawn with a sterile, dry syringe. A small drop of the fluid was placed on a plastic cover slip and inverted on a well slide which had been ringed with vaseline to prevent entrance of air. Preparations were examined immediately on a warm stage under oil immersion. For most of the preparations no anesthesia was used. However, when the abdomen was incised, occasionally at forty-eight hours, more often at the end of ninety-six hours or a longer interval after injection of organisms, ether anesthesia was given.

Guinea pigs. The same procedure as for mice was used except that larger amounts of materials were injected. Ten milliliters of 7.2 per cent sodium caseinate were injected intraperitoneally on two successive days, followed in six hours after the last injection by 0.5 ml. of stained organisms suspended in 10 ml. of aqueous buffer solution (pH 7.0).

RESULTS

Phagocytosis Experiments with Stained Organisms

Mice—brom phenol blue: Observations on a typical experiment undertaken on a mouse weighing 28 gm. and injected intraperitoneally with *M. tuberculosis* H37Rv stained with brom phenol blue (yellow-pH 3.0, blue-pH 4.6) are presented.

Two hours after the injection of organisms, a small amount of fluid was aspirated from the peritoneal cavity. In a single field 4 to 5 neutrophils and an occasional monocyte with phagocytized organisms, stained intensely blue, were

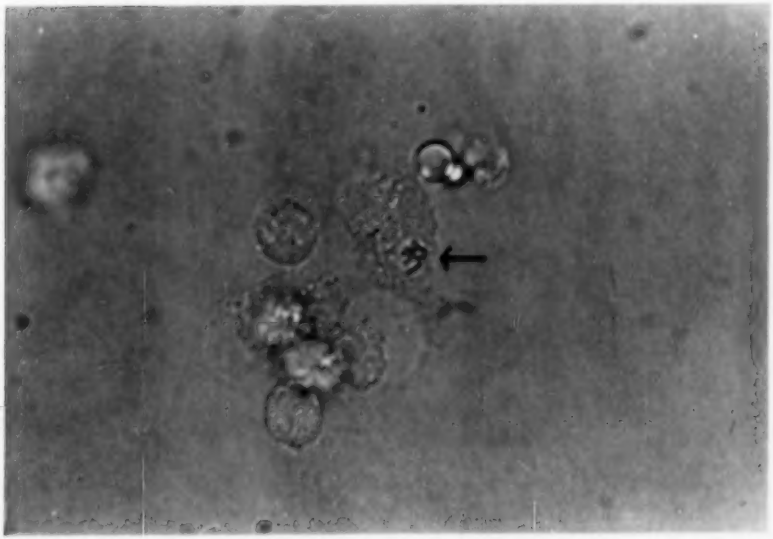

FIG. 1. Phagocytosis by monocyte of mouse of H37Rv stained with brom phenol blue. Bacilli (arrow) were blue—color of basic range of indicator. Original colored photomicrograph taken five and one-half hours after intraperitoneal injection. (× 980)

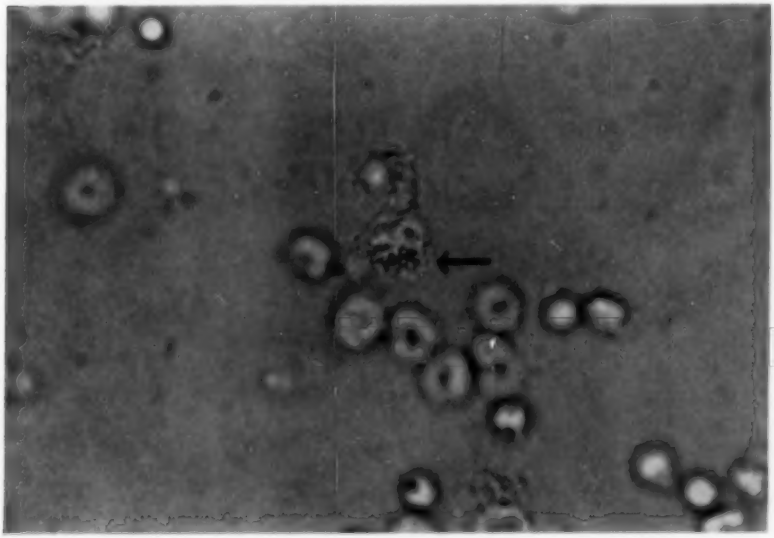

FIG. 2. Phagocytosis by monocytes of mouse of *M. smegmatis* stained with brom phenol blue. Cells shown are from nodule on large intestine. Organisms (arrow) were blue (colored photomicrograph) forty-eight hours after intraperitoneal injection. (× 980)

observed with the oil immersion objective (×735). The extracellular organisms were also blue. After twenty-four hours more monocytes had phagocytized blue-stained organisms, and some neutrophils with ingested brilliant blue organisms had been engulfed by monocytes. No color was noted in the normal cell granules. At seventy-two hours the color of the bacilli ingested by the monocytes was still blue, showing no evidence of shifting to the yellow or acid range of the dye. Few neutrophils and no extracellular organisms now were observed. Under the conditions of the experiment there was no way of knowing just how long the ingested bacteria had been in any particular cell. However, it appeared that, since no extracellular organisms were found after forty-eight or seventy-two hours following injection, the engulfed bacilli had probably been phagocytized for some hours.

In another experiment a photomicrograph of cells from aspirated peritoneal fluid was taken five and one-half hours after intraperitoneal injection of *M. tuberculosis* H37Rv stained with brom phenol blue (figure 1). It will be noted that a clump of five or six organisms has been ingested by a large monocyte. The bacilli, as shown in the original color photograph, retained the blue color of the basic range of the indicator. A suggestion of a vacuole can be observed about the phagocytized bacteria. The argument may be advanced that the organisms had not been in the cell long enough for sufficient acidity to develop to change the color of the bacilli. However, a phagocytic cell (figure 2) is shown which was taken from a nodule on the large intestine forty-eight hours after the stained

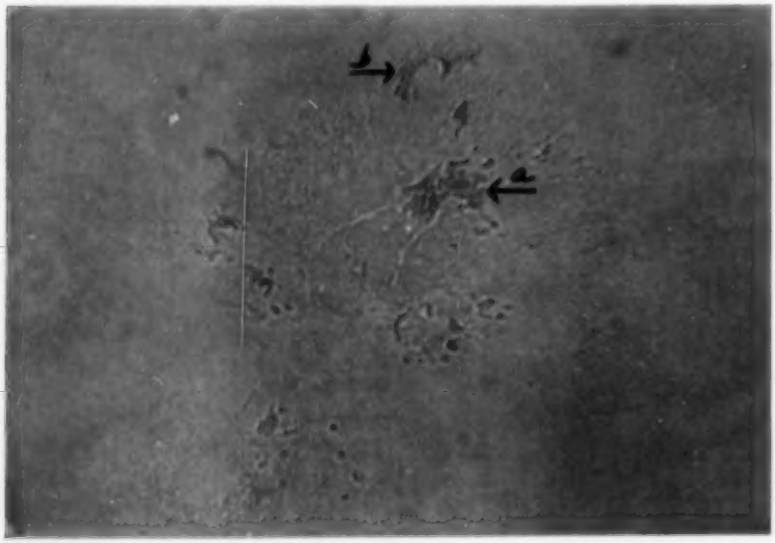

FIG. 3. Monocytes of a mouse eighteen hours after intraperitoneal injection of H37Rv stained with brom cresol green. Colored photomicrograph showed blue-green extracellular bacilli (arrow *a*). Phagocytized organisms (arrow *b*) appeared yellow-green. (× 980)

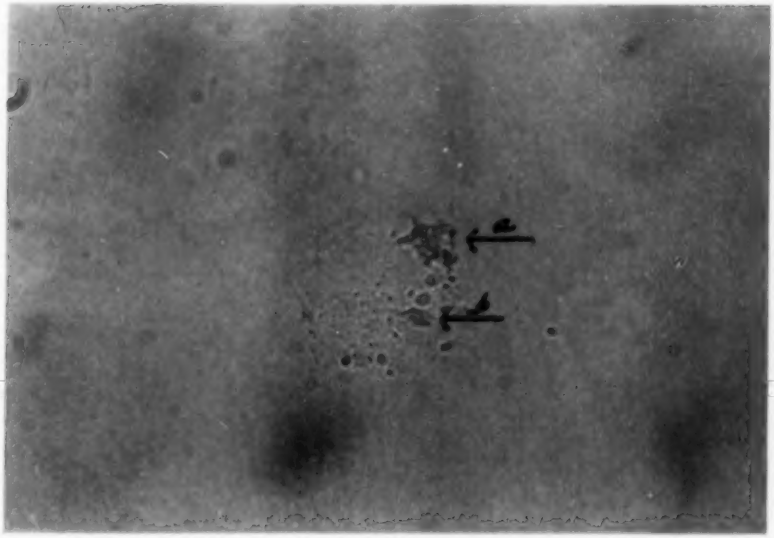

FIG. 4. Monocyte of mouse containing H37Rv stained with brom cresol green. Large clump of bacilli (arrow *a*) was green, whereas smaller group was greenish yellow (arrow *b*). Original colored picture was taken eighteen hours after injection. (× 980)

bacteria, in this case *M. smegmatis*, had been phagocytized. The bacteria in the monocyte were definitely blue after this period of time.

Mice—brom cresol green: The cells of a mouse eighteen hours after injection with brom cresol green (yellow-pH 3.8, blue-pH 5.4) are shown in figures 3 and 4. The extracellular organisms clumped in the center were blue-green (figure 3) whereas some of the bacteria ingested by the large monocytes directly above the extracellular bacteria and also those in the cell farthest to the left were green with a tinge of yellow. The pK value which is the point of 50 per cent dissociation of the indicator is 4.7 for brom cresol green. This would suggest that the ground matrix surrounding the engulfed bacteria in the monocyte may possibly be as low as pH 4.7. However, no cells were observed with lemon-yellow organisms which would indicate a pH of 3.8 or lower. Two clumps of phagocytized organisms were observed in a monocyte (figure 4). The larger group was green, whereas the smaller clump had a greenish-yellow appearance. The large refractile granules had a green tinge.

Guinea pigs—brom phenol blue: A photomicrograph of cells from the peritoneal fluid of a male guinea pig was taken twenty-six hours after *M. tuberculosis* H37Rv stained with brom phenol blue was injected into the peritoneal cavity. Large monocytes with as many as 3 or 4 phagocytized neutrophils were observed (figure 5). The bacteria engulfed by the neutrophils which in turn had been phagocytized by the monocytes retained the blue color of the indicator and did

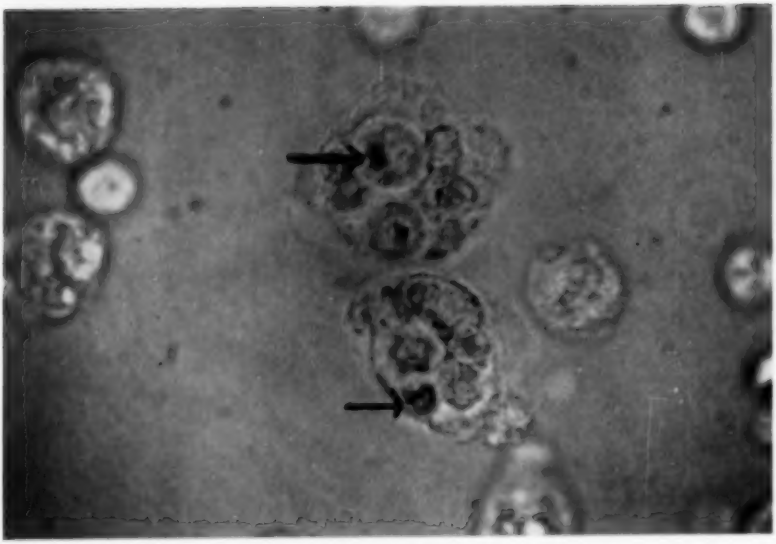

FIG. 5. Monocytes from peritoneal fluid of guinea pig containing neutrophils with phagocytized blue-stained organisms (arrow). Original colored picture was taken twenty-six hours after injection of H37Rv stained with brom phenol blue. (× 980)

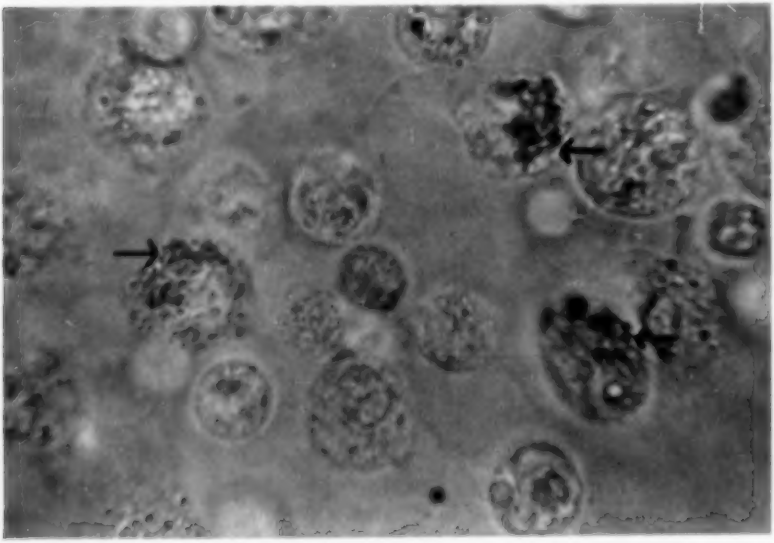

FIG. 6. Original colored photomicrograph taken forty-eight hours after intraperitoneal injection of H37Rv stained with brom phenol blue into a guinea pig showed monocytes with numerous phagocytized bacilli (arrow) stained blue. (× 980)

not change to yellow, the acid range of the dye. In figure 6, taken forty-eight hours after the organisms had been injected, the bacteria which can be observed in the monocytes were intensely blue. After seventy-six hours no extracellular blue-stained bacteria were found, but blue-colored organisms were found in the monocytes and neutrophils although there were fewer monocytes with phagocytized neutrophils than at twenty-six and forty-eight hours.

Guinea pigs—brom cresol green: A male guinea pig was injected intraperitoneally with *M. tuberculosis* H37Rv stained with brom cresol green. After twelve hours, cells in the fluid aspirated from the peritoneal cavity were photographed. A monocyte with engulfed bacteria is shown in the center of the field (figure 7). These bacilli were blue-green.

It was thought that there might be a difference in the degree of acidity produced in the monocytes of the mouse and those of the guinea pig. Grogg and Pearse (2), using histochemical techniques, had found that the mononuclear phagocytes of the mouse contained a strong acid phosphatase while none could be demonstrated in the monocytes of the susceptible guinea pig. The organisms stained with brom cresol green, phagocytized by the monocytes of the guinea pig, were blue-green while those phagocytized by the monocytes of the mouse were green sometimes with a yellow tinge. However, under the experimental conditions employed, the slight differences in the color of the phagocytized organisms were not sufficient to indicate that the acidity about phagocytized organisms in the monocyte of the mouse was a lower pH than in the monocyte of the guinea pig.

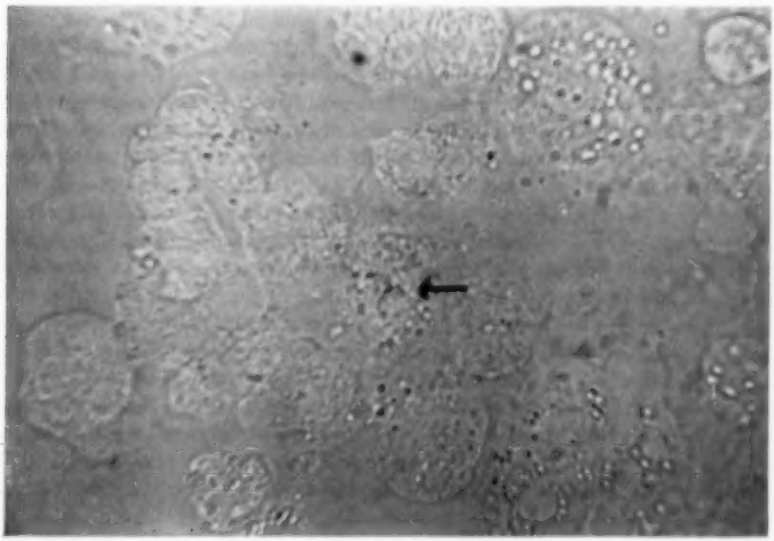

FIG. 7. Original colored photomicrograph taken twelve hours after intraperitoneal injection of H37Rv stained with brom cresol green into a guinea pig showed a monocyte with engulfed bacteria (arrow) stained blue green. (× 980)

Examination of peritoneal cavities: To ascertain whether the color of the phagocytized organisms would change after being ingested for a longer period of time, animals were subjected to ether anesthesia and the peritoneum was incised. Seven days after a guinea pig was inoculated intraperitoneally with *M. tuberculosis* H37Rv stained with brom phenol blue, the animal was anesthetized with ether. The abdomen was incised while the animal was living. Frequently, before the peritoneum was opened, small blue nodules could be observed underneath the peritoneal lining. Since, on exposure to air, the tissue of animals injected with vital stains may take on a more intense hue, as Rous (1) noted, care was taken to make a very small incision in the peritoneal lining. Many intensely blue nodules on the intestine, the omentum, and an occasional one on the liver and spleen were observed. There was no evidence of any yellow coloration, indicating an acid reaction, at the periphery of the colored tissue. A nodule was quickly excised, gently crushed under a plastic cover slip, and examined microscopically. The reaction at the center of the nodule, using universal indicator paper (a qualitative procedure), was pH 7.0. Many monocytes and neutrophils exhibited blue-stained bacilli in the cells. Similar findings were observed in guinea pigs injected with organisms stained with brom cresol green and in mice injected with organisms stained with brom phenol blue and brom cresol green.

Guinea pig—brom cresol purple: Since the pK values of brom phenol blue and brom cresol green are 4.0 and 4.7, respectively, it was thought it would be of interest to use an indicator such as brom cresol purple, which is yellow, at a pH 5.2 and purple or blue at a pH 6.8. The pK value of this indicator is 6.3, which is the point of maximal intensity of both yellow and purple or a yellow-green color.

An experiment using *M. tuberculosis* H37Rv stained with brom cresol purple was as follows: Six hours after the bacilli were injected intraperitoneally into a male guinea pig, a very small amount of colorless fluid was obtained from the peritoneal cavity. Many monocytes showed engulfed bacteria which were yellow-green. A number of monocytes with ingested neutrophils containing yellow-green organisms also were found. At twenty-four hours a small amount of colorless fluid was withdrawn which had a pH of 7.0. At this time the color of the phagocytized bacilli within the monocytes was yellow with just a tinge of green. A small amount of N/20 sodium hydroxide was run under the cover slip, and the bacilli in the cells became pale blue after a period of ten to fifteen minutes. After forty-eight and ninety-six hours, monocytes containing organisms which appeared to be yellow, tinged with green, were observed. Six days after injection of stained organisms, the animal was placed under ether anesthesia, and the abdomen was incised. The scant amount of peritoneal fluid contained monocytes with engulfed bacteria stained yellow-green. The omentum and spleen revealed many small yellow-green nodules. Organisms in cells of the nodules were of the same intense color as those found in the cells of the free fluid. These phagocytized yellow-green bacilli also changed to blue, the basic color of the indicator, when a small amount of N/20 sodium hydroxide was added underneath the cover slip.

The results obtained with phagocytized organisms stained with sulfonphthalein indicator dyes did not conform with expected findings. There was no indication

that the reaction about the stained bacilli became as low as pH 3.0 in either the monocytes of the mouse or guinea pig. It was therefore deemed advisable to reinvestigate the injection of sulfonphthalein dyes into mice.

Intraperitoneal injections with the phthalein dyes: Solutions of brom phenol blue and brom cresol green were injected into white mice in order to ascertain whether the acid material held in the cytoplasm in granular form (1) could be observed. The dyes were prepared according to the methods outlined under experimental methods. The experiments were repeated a number of times. Rous (14) described two types of granules in the macrophage. One type was the highly refractile granule like those found in the polymorphonuclear cell and the other was a non-refractile, relatively small translucent granule. He found that it was the latter type of granule in which the acid vital dyes became stored.

Brom phenol blue (yellow at pH 3.0, blue at pH 4.6): Rous (1) injected a mouse weighing 27 gm. with 1 ml. of a 1 or 2 per cent solution of brom phenol blue in 0.9 per cent saline for three successive days and aspirated peritoneal fluid twenty-four hours after the last injection. Since, in the experiments to be reported, it was found that a 1 ml. dose was too toxic for the mice, 0.5 ml. was injected intraperitoneally for six successive days, a total of 3 ml. being given. Within three or four minutes after the dye was injected, the tail became tinted blue, and gradually the eyes, ears, and skin all became a vivid blue. The blue color was present for eight to twelve hours but had disappeared in twenty-four hours, or by the time the next injection was given. The urine and feces were blue.

Since the phthalein dyes are so easily assimilated by the animal, it was thought that, if the fluid was aspirated from the peritoneal cavity at intervals of less than twenty-four hours after the dye was administered, it might be possible to detect the dye in the cells. Aspiration of fluid from the peritoneal cavity was made one hour after the injection of 0.5 ml. of the dye. At this time there did not appear to be any increase in the normal cell granules in either the neutrophil or monocyte. However, clustered about the normal refractile granules were very fine, minute, blue particles. Six hours after the fourth injection a very small amount of peritoneal fluid was withdrawn. The ears, eyes, and tail of the mouse were still blue. There were now many large refractile granules in the cells which had a greenish blue cast. No fine blue particles were observed nor were any bright yellow ones found. In some monocytes small globules of blue dye, possibly a coalescing of fine particles, were observed. Twenty-four hours after the fourth injection, the scant amount of peritoneal fluid aspirated revealed a number of monocytes, but no fine, non-refractile lemon-yellow granules were found. Twenty-four hours after the sixth injection of dye, the mouse appeared normal. The animal was anesthetized with ether and the abdomen was incised. There was a small blue nodule on the peritoneal wall and a very small amount of slightly bloody exudate. Neither the cells of the exudate nor those from the nodule showed any yellow non-refractile granules.

Brom cresol green (yellow at pH 3.8, blue at pH 5.4): A 27 gm. mouse was injected with 0.4 per cent of a 1 per cent solution of brom cresol green for six successive days. After each injection of the dye, the skin, tail, ears, eyes, and

feet all appeared a vivid green. The color persisted for six to eight hours but had entirely disappeared in twenty-four hours or by the time the next injection was given. Fluid was aspirated from the peritoneal cavity at the following intervals: two hours after the second injection, six and twenty-four hours after the fourth, and twenty-four hours after the sixth injection of dye. At these time intervals no fine non-refractile granules stained lemon-yellow were observed in the monocytes.

DISCUSSION

The experiments described and illustrated in figures 1 to 7 were made in an attempt to determine whether organisms stained with indicator dyes and phagocytized by the neutrophils and monocytes of the mouse and guinea pig would shift to the acid range of the dye, indicating a relatively high hydrogen-ion concentration surrounding the ingested bacteria.

It is generally believed that phagocytized material ingested by neutrophils and monocytes is aggregated in a vacuole, the reaction of which is not the same as the cytoplasm (15–17). Metchnikoff (15) thought that the digestion of the phagocytized bacteria was aided by digestive ferments and that it took place in a vacuole, the fluid of which was weakly acid. No vacuoles were seen around the phagocytized bacteria stained with indicator dyes when observed under the microscope. In figures 1, 3, and 4 there is a suggestion, however, that there is a clear area, perhaps a vacuole, surrounding the engulfed organisms.

Chambers (18) found that the color of intracellular inclusions of amebae could be shifted by a change in the environment, exposure to either carbon dioxide or ammonia, but that the pH of the cytoplasmic matrix remained constant. There is some evidence that the environment surrounding the stained phagocytized organisms may be slightly different from the cytoplasm, since the color of the organisms became more intense when slides were not ringed with vaseline and thus were exposed to air.

If the bacilli are aggregated in a vacuole, a reaction with the indicator molecule adsorbed to the organisms might take place with greater facility than if the microorganisms were free in the cytoplasm. Frey-Wyssling (19) has noted that the end groups of organic compounds which are constituents of the vacuolar colloids are not screened off as in the cytoplasm and are therefore reactive. However, the acid and basic groups of the molecular framework which are screened off in the cytoplasm must first be liberated by slight hydrolysis in order to be capable of reacting with the dyestuff.

Whatever end groups of organic compounds may have been present in the material surrounding the ingested bacilli, they did not appear to shift the indicator stain adsorbed to the organisms completely to the acid side. According to Conn (9), "these color changes in the sulfonphthalein indicators are generally assumed to be due to alterations in the structure of the molecule such as the disappearance and reappearance of the quinoid ring, but the relation of structure to color is complicated and has not yet been worked out to general satisfaction." Therefore, no theory as to what groups present in the material surrounding the stained organisms might react with the indicator molecule has been advanced.

Whether the stained organisms were surrounded by a vacuole or were in the

cytoplasm, they apparently did not elicit an acidity as low as pH 3.0, since the organisms stained with brom phenol blue were definitely blue after being phagocytized by the monocytes and neutrophils and were not even slightly changed to green or greenish yellow. Bacilli stained with brom cresol green and colored blue when injected became green, sometimes green tinged with yellow, when observed in the monocyte and neutrophil twenty-four hours after intraperitoneal injection. It should be noted that the gradation in color from green to yellow-green to yellow tinged with green is subject to visual acuity, and the relation between the color observed and pH determination is a qualitative measurement. However, since no color change was observed in the phagocytized bacteria stained with brom phenol blue, and a shift from the basic range to the slightly acid range was observed with brom cresol green and brom cresol purple, it would appear that the relative hydrogen-ion concentration about the phagocytized bacilli in the monocyte of the normal mouse and guinea pig was in the pH range 4.7 to 5.5.

It is of interest that the observed pH range 4.7 to 5.5 in the environment immediately adjacent to the stained phagocytized tubercle bacilli is relevant to the findings with antituberculous drugs. Since it is known that tubercle bacilli can survive in an acid environment, it is not probable that the intracellular pH of the phagocytic cells has any direct bactericidal effect on the mycobacteria. Dubos (20) has stated: "The hypothesis that intracellular acidity is one of the causes of the bactericidal effect of phagocytosis is at best a working hypothesis almost devoid of experimental support."

However, in the drug treatment of tuberculous infections, it may be that the intracellular acidity effects a change in drug–tubercle bacilli–host relationships. Thus, the observed pH range 4.7 to 5.5 surrounding the intracellular stained mycobacteria, although not as low a pH as expected, is sufficient to produce a condition which may affect the activities of the drugs. Streptomycin shows a marked reduction of antimicrobial activity at pH 5.5 whereas nicotinamide and pyrazinamide *in vitro* exhibit greatest activity in a pH range of 5.0 to 5.5 (4), and also show activity against intracellular mycobacteria in monocytes (8). Isoniazid shows no gain or loss in acidic environments.

It appears that the acid reaction observed by Rous (1) in the macrocyte of the mouse was of relatively short duration, as colored granules were found in twenty-four but not in forty-eight hours after the last injection of the dye. The total amount of dye injected in the present study was the same as that used in the original work except that smaller doses of brom phenol blue (0.5 ml. for six successive days, rather than 1.0 for three days) were administered. Four-tenths of a milliliter of 1 per cent brom cresol green for six successive days was injected rather than 0.4 ml. of 2 per cent solution for two days. When 1.0 ml. amounts of 2 per cent solutions were used, the animals died. It is possible that, due to smaller doses, the animal assimilated all of the dye and none was available for storage in the granular form.

SUMMARY

M. tuberculosis var. *hominis* H37Rv and *M. smegmatis* were stained with sulfonphthalein dyes, brom phenol blue, brom cresol green, and brom cresol purple.

The stained organisms were then injected intraperitoneally into mice and guinea pigs which had received sodium caseinate to promote production of phagocytic cells. The phagocytized mycobacteria were examined at intervals under the microscope for evidence of pH change. Under the experimental conditions employed, the environmental acidity surrounding phagocytized organisms stained with indicator dyes in the monocyte and neutrophil of the mouse and guinea pig was in the pH range 4.7 to 5.5.

No difference in the reaction about phagocytized organisms in the mononuclear phagocytes of the mouse and guinea pig was detected.

The development of an acidity as low as pH 3.0 was not observed about phagocytized stained organisms or in either the monocyte or neutrophil of the adult white mouse when injected intraperitoneally with 1 per cent solutions of the indicator dyes, brom phenol blue and brom cresol green.

Acknowledgment

The writer is indebted to Dr. Walter Mack and Dr. Russell Gottshall for their direction throughout the study.

SUMARIO

Fagocitosis del M. Tuberculosis y del M. Smegmatis Teñidos con Colorantes Indicadores

M. tuberculosis var. *hominis* H37Rv y *M. smegmatis* fueron teñidos con colorantes de sulfoneftaleina, azul de bromfenol, verde de bromcresol y púrpura de bromcresol. Los microbios teñidos fueron luego inyectados intraperitonealmente en ratones y cobayos que habían recibido caseinato de sodio para fomentar la producción de fagocitos. Las micobacterias fagocitadas fueron estudiadas a ciertos intervalos al microscopio en busca de signos de alteraciones del pH. En las condiciones experimentales empleadas, la acidez del ambiente que rodeaba a los microbios fagocitados teñidos con colorantes indicadores en los monocitos y neutrófilos del ratón y del cobayo quedaba entre los límites de 4.7 a 5.5 del pH.

No se descubrió diferencia alguna en la reacción alrededor de los microbios fagocitados en los fagocitos mononucleares del ratón y del cobayo.

No se observó la aparición de acidez de no más de un pH de 3.0 alrededor de los microbios teñidos fagocitados o en los monocitos o neutrófilos de ratones blancos adultos cuando se inyectaban intraperitonealmente soluciones al uno por ciento de los colorantes indicadores, azul de bromfenol y verde de bromcresol.

RESUME

Phagocytose de M. tuberculosis et de M. smegmatis colorés avec des colorants indicateurs

Le *M. tuberculosis* var. *hominis* H37Rv et *M. smegmatis* furent colorés avec des colorants à la sulfophtaléine, bleu brom-phénol, vert brom-crésol et violet brom-crésol. L'injection intrapéritonéale des bacilles colorés fut alors pratiquée chez des souris et des cobayes traités avec du caséinate de sodium pour provoquer la production de cellules phagocytes. Les mycobactéries phagocytées ont été examinées périodiquement au microscope pour déceler les variations du pH. Dans les conditions de l'expérience l'acidité ambiante autour des bacilles phagocytés colorés avec les colorants indicateurs dans le monocyte et le neutrophile de souris et de cobaye variait entre pH 4,7 et 5,5.

Il n'a été décelé aucune différence dans la réaction autour des bacilles phagocytés dans les phagocytes mononucléaires de la souris et du cobaye.

L'acidité à pH 3 n'a été atteinte autour des bacilles colorés ayant été phagocytés ni dans le monocyte ou le neutrophile de la souris blanche adulte à la suite de l'injection intrapéritonéale avec les solutions à 1% des colorants indicateurs, bleu brom-phénol et vert brom-crésol.

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THE INFLUENCE OF TETRACYCLINE COMPOUNDS ON THE DEVELOPMENT OF ANTIBODIES IN PSITTACOSIS[1]

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INTRODUCTION

The diagnosis of the illness of patients with histories of exposure to birds with psittacosis, by means of the customary laboratory procedures and physical and roentgenographic evidence, is becoming more difficult. Rarely are efforts made to isolate the infective agent from the blood or sputum, and these efforts cannot be expected to succeed if the patient has been given early and intensive drug treatment before the appropriate diagnostic specimens are collected. In many instances convalescent or postconvalescent serum cannot be obtained for completion of the serologic study and serodiagnosis is doubtful without comparison of results. Until the last few years it was commonly thought that serodiagnosis could be made when complement-fixing, agglutinating, or neutralizing antibodies appeared and increased in titer during convalescence, but now this must be reconsidered.

Early and prolonged intensive treatment with tetracycline compounds may delay, decrease, or completely suppress antibody production (1–5). These reports stress that the delay in the antibody response may render serodiagnosis difficult. The titer early in convalescence may be only 1:10 or 1:20, titers which are fairly common in the serum of the healthy.

In 4 cases of infection with a psittacosis agent, proved through isolation of the agent, the patients were treated with tetracycline compounds, and the complement-fixation titers were studied early in the illness, throughout convalescence, and several months later.

OBSERVATIONS

Case 1. A 32-year-old man was exposed to a parakeet known to be infected with psittacosis. Twelve days after the bird died the man had chills, severe headache, and a temperature of 102.8° F. A blood sample was taken on the day of onset. The complement-fixation test was negative, but a parakeet psittacosis agent was isolated from this blood sample on the second mouse passage. The patient was treated with 4 gm. of tetracycline daily for four days and with 2 gm. a day for six days. The response was excellent and recovery was complete, beginning thirty-six hours after the initiation of treatment. Repeated complement-fixation tests on the twenty-fifth, fortieth, fifty-sixth, and one hundred and thirty-second days were all negative.

Case 2. The patient, a 52-year-old man, was also exposed to an infected parakeet. The onset of the illness was typical and pulmonary involvement developed. On the second day of the illness a blood sample was taken and a psittacine agent was isolated from it by

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mouse passage. Treatment consisted of chlortetracycline, 2 gm. daily for ten days, and the response was good. The complement-fixation titer was 1:2 on the eighteenth day, but on the thirty-eighth day it was found to be 1:4. It was still 1:4 when the last test was made, eighty days after the illness began.

Case 3. Initial symptoms in this patient, a woman, were typical of psittacosis with pulmonary involvement. The patient believed that, of possible sources of infection, she had been exposed only to ducks. An ornithosis agent of low virulence was isolated from a blood specimen taken before treatment. Chlortetracycline was given in daily doses of 2 gm. for two days and 1 gm. for six days. The complement-fixation reaction was still negative in the blood sample taken on the one hundred and forty-fourth day, but it was 1:4 in the hemolyzed serum from a sample taken on the one hundred and eighty-eighth day.

Unfortunately, as usual, the clinical report to the laboratory is somewhat sketchy, but it is known that the son of this woman who had played with the ducks had pneumonia (left lower lobe) about the time of his mother's illness. The complement-fixation titer in his serum collected one hundred and eighty-five days after his mother's illness was 1:8.

Case 4. An animal caretaker, 60 years old, was exposed to guinea pigs experimentally infected with bovine encephalomyelitis virus, an agent antigenically related to the psittacosis group. For two days he complained of tiredness, of not feeling well, and of headache. When he consulted his physician on the third day of symptoms his temperature was 101° to 102° F., and he had rales at the base of the left lung. A blood sample was taken at this examination, and the bovine encephalomyelitis virus was isolated from it. Intensive treatment consisted in 4 gm. of chlortetracycline daily for six days and 1 gm. for seven days. The patient was afebrile within forty-eight hours after treatment was begun, and his recovery was uneventful. The complement-fixation titers with yolk sac psittacosis antigen were 1:8 on the second day, 1:64 on the tenth day.

In these 4 cases in which tetracycline compounds were given orally to patients with proved viremia and pulmonary localization, specific antibody production was less evident than it usually is in the untreated disease. In *Case 4* the dose for the first four days was twice the usual dose (2 gm.), and in the remaining 3 cases it was within the usual daily dose range. Isolation of the infective agent proved the nature of the illness in all cases. The complement-fixation titers reached diagnostic levels in one case, and one cannot be sure that they were not suppressed in this case.

Comment: According to a recent report (4), another patient whose serum had shown no complement-fixation reaction on or before the thirtieth day had a high titer on the one-hundred and sixth day after onset and intensive therapy.

The epidemiologic and clinical evidence in all of these cases was suggestive, but in only one would the diagnosis have been corroborated by the serum findings. One aspect of the titers does differentiate them from anamnestic reactions: they were low, but they did persist and in some cases rose very late in convalescence. In anamnestic reactions the titers are highest during the acute phase, but drop and disappear early in convalescence. If the patient had been infected previously with any member of the psittacosis-lymphogranuloma group, complement-fixing antibodies against this group might be demonstrable during some other infection later. In this case the results of the first complement-fixation test may

be positive, but within ten to fourteen days when a second specimen is examined they may be negative.

The absence of complement-fixing antibodies in cases in infants in which the infective agent was isolated before treatment is not clearly understood. It cannot be explained by treatment because, in most of the cases, there was no clinical response to treatment (6).

Antibody production may not be delayed or completely suppressed in all cases in which the tetracycline compounds are used (*Case 4*). In all infections the amount of antigen released by the infector varies, and it is the amount of antigen which to a large degree determines the amount of antibody produced. It is not yet known whether the antibody depression is secondary to low antigen stimulus or to direct depression of antibody production. The few carefully conducted experimental studies on mammals have shown that, when chlortetracycline, oxytetracycline, or chloramphenicol is administered parenterally or orally, cellular antibody production is in some way modified or completely inhibited (7). Penicillin may also affect the immune response (7, 8).

* * *

In preliminary tests in this laboratory 2 primates were given an intravenous injection of a relatively large dose of psittacosis virus (0.5 ml. of virus with a titer of $LID_{50}:10^{-5}$) and one was then treated with daily oral doses of 75 to 150 mg. of chlortetracycline. Antibody production was not depressed, for on the first day of therapy (the second day after infection), complement-fixing antibodies in the titer of 1:4 were demonstrable. The titer rose to 1:256 within six days and remained at that level on the fifteenth day after infection. On the twenty-second day the titer of the treated monkeys had dropped to 1:128, while that of the untreated animals remained at 1:256. The explanation offered is that the widespread imprint of the intravenously injected antigen on the cell was intense before the drug reached it. Antibody formation had already been stimulated before the agent and antigen were destroyed.

Incidental observations in the course of pilot studies of the effects of tetracycline therapy on psittacosis in parakeets showed that under certain circumstances antibody production is partially or completely suppressed in this host.

A group of 100 parakeets artificially infected with a moderately virulent strain of psittacosis agent were proved to be infected by the eighteenth day. Four had died of acute psittacosis, and the complement-fixation reaction was positive in 2 of 10 birds examined for this purpose. Twenty-four infected birds (*Group 1*) were treated orally with medicated water (0.85 mg. of oxytetracycline per milliliter of water), an average total intake of 29 mg. of oxytetracycline per bird in fifteen days. *Group 2* was given 5 mg. of tetracycline in an oil adjuvant by intramuscular injection on the first and fifth days, a total of 10 mg. per bird, and *Group 3* was fed for fifteen days on medicated feed containing 1.87 mg. of chlortetracycline per gm. of millet seed, an estimated total drug intake of 145.6 mg. per bird.

When sacrificed twelve days after the termination of therapy, 45 per cent of the birds in *Group 1* showed the residuals of infection (enlarged spleen), but only one had a complement-fixation titer (1:16) (figure 1). Repeated subinoculation of mice proved the organs of the birds to be free from the psittacosis agent.

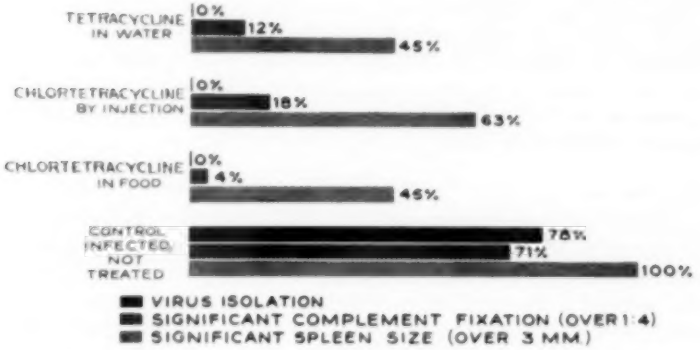

FIG. 1. Antimicrobial therapy in parakeet psittacosis (summary).

Nearly identical observations were made on the other two groups. Of the infected untreated parakeets, 78 per cent still carried the virus, and the sera of 71 per cent gave complement-fixation reactions in dilutions of 1:4 and more. The suppressive effect of the antimicrobials ten to twelve days after completion of treatment was most marked in *Group 3*, which had received the largest amount of drug. Antibodies were demonstrable in nearly a fifth of *Group 2*, treated with doses comparable with those used in man. Although experimental findings in birds cannot be applied directly to the human disease, they can serve as guides for future studies.

GENERAL COMMENT

Early and intensive treatment with large doses of antimicrobial drugs ensures quick clinical response, but may not allow for the development of antibodies. But, if the usual daily dose (30 mg. per kg.) is given for only twelve days, it should, in the light of the observations on birds, be curative and only temporarily impede the formation of antibodies so important in the diagnosis of the disease.

The clinician who has to interpret the complement-fixation reaction in the diagnosis of human psittacosis must remember the following:

The reaction is subject to wide individual variations even in the acute phase of the disease (4, 9).

A definite rise in titer must be interpreted with the clinical course.

A diagnostically significant titer may not be reached until the thirtieth, even the one hundred and eightieth, day of convalescence if the illness is treated with tetracycline compounds.

The titer may persist unchanged for months after the illness.

The duration of a positive complement-fixation reaction after the disease has run its course seems to differ from case to case (4, 6).

Anamnestic reactions are not infrequent (3–5). They will rise during the acute phase and drop during convalescence.

Tetracycline compounds in the low dose of 1.5 gm. daily, although curative, may not influence the antibody titer (10).

Whether the physician should make a diagnosis, even though the titer is low, when a patient with avian exposure has a clinical course characteristic of psittacosis and shows prompt response to appropriate therapy requires further and more detailed study (11).

SUMMARY

Now that patients are being treated for psittacosis early in the infection, it is more important than ever that diagnostic material (blood and sputum) be collected before treatment is begun. The antibody response in psittacosis may be weak and delayed if the infection is treated with large doses of the tetracycline compounds, so that it is still necessary to study the serologic results well into convalescence. A slightly lower dose (1.5 gm. per day) might control the infection but allow the development of specific antibodies.

SUMARIO

El Influjo de los Compuestos de Tetraciclina sobre la Formación de Anticuerpos en la Psitacosis

Hoy día, que ya se trata a los enfermos por psitacosis en los comienzos de la infección, es más importante que nunca recoger material de diagnóstico (sangre y esputo) antes de iniciar el tratamiento. En la psitacosis, la respuesta en anticuerpos tal vez sea débil y tardía si se trata la infección con dosis grandes de los compuestos de la tetraciclina, de modo que es todavía necesario estudiar los resultados serológicos, aun entrada la convalecencia. Una dosis un poco más baja (1.5 gm. al día) podría dominar la infección y permitir la formación de anticuerpos específicos.

RESUME

L'influence des composés de tétracycline sur le développement des anticorps dans la psittacose

Maintenant que les malades atteints de psittacose sont traités dès le début de l'infection, il est plus important que jamais de prélever le matériel de diagnostic avant de commencer le traitement. Dans la psittacose la réponse anticorps peut être faible et tardive quand l'infection est traitée avec les composés de tétracycline à dose forte, de telle sorte que l'étude des résultats sérologiques doit se poursuivre assez longtemps au cours de la convalescence. Une dose légèrement plus faible (1,5 g par jour) pourrait juguler l'infection, tout en permettant le développement d'anticorps spécifiques.

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RESISTANCE OF TUBERCLE BACILLI TO PYRAZINAMIDE IN VIVO[1, 2]

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INTRODUCTION

The nicotinamide derivation, pyrazinamide, was first shown by Malone and his associates (1) to be active in the treatment of tuberculosis in mice. In the first description of the effect of this drug in humans, Yeager and co-workers (2) noted that relapses of acute tuberculous disease sometimes occurred as early as six weeks after institution of therapy. This loss of effectiveness of pyrazinamide was assumed to be due to the emergence of drug-resistant strains of tubercle bacilli in the patients under treatment, and in these initial studies (2, 3) *in vitro* tests appeared to corroborate this assumption. Subsequent studies, however, failed to confirm the original observation that wild-type tubercle bacilli are inhibited by pyrazinamide *in vitro* (4, 5). This lack of demonstrable *in vitro* activity obviously made it impossible to correlate apparent loss of *in vivo* activity with the emergence of drug-resistant strains.

In a previous report from this laboratory (6) it was noted that, although pyrazinamide was inactive against tubercle bacilli *in vitro* at pH 7.0, it became quite active in inhibiting tubercle bacilli of human origin when tested at pH levels in the range 5.0 to 5.5. The significance of the *in vitro* test at these low pH levels could only be surmised. That it might have actual significance with reference to *in vivo* drug action was suggested by the additional observation that bovine tubercle bacilli which were natively insusceptible to pyrazinamide *in vivo* (7) were likewise not inhibited by the drug *in vitro* even at a low pH (8).

During the course of an experiment in mice originally infected with *M. tuberculosis* H37Rv, a strain of tubercle bacilli was isolated from an animal which had had twelve weeks of treatment with pyrazinamide. The population of tubercle bacilli in the lungs of this animal was at a relatively high level even though, at two previous samplings of the animal group to which it belonged, the populations of tubercle bacilli in the tissues had all been quite low. This suggested that a resurgence of the tubercle bacilli had occurred in the animal despite the continued administration of pyrazinamide and that the strain in question represented a pyrazinamide-resistant derivative of the drug-susceptible parent strain H37Rv.

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[3] With support from Sigrid Juselius Foundation and the University of Helsinki, Finland.

[4] James Alexander Miller Fellow of the New York Tuberculosis and Health Association, Inc.

Accordingly, the strain of tubercle bacilli isolated from the animal and designated strain E11L was subjected to special studies *in vitro* and *in vivo*. The results of these studies form the basis of the present report.

MATERIALS AND METHODS

Details of the methods employed have been described elsewhere (6, 9-11). *In vitro* testing was performed in Tween®-albumin and oleic acid-albumin media. Two strains of *M. tuberculosis* were used, the stock laboratory strain H37Rv and the strain E11L, the derivation of which has been described above. Inocula were prepared from a seven-day growth in Tween-albumin medium and consisted of 0.02 ml. of culture or approximately 1×10^6 viable units of tubercle bacilli inoculated into each 5 ml. volume of medium. Adjustment of the two media to the desired pH was made with sodium hydroxide or hydrochloric acid. Cultures were maintained at 37°C. and were inspected weekly for two weeks. The values were recorded as the amount of macroscopically visible growth after two weeks on an arbitrary scale of 0 to 4 plus.

The technique of determining the total population of tubercle bacilli in tissues has been described in detail elsewhere (9, 10) and was briefly as follows:

> Large groups of mice were infected by intravenous inoculation of tubercle bacilli and sample groups were sacrificed at intervals thereafter. Lungs and spleens were measured and homogenized, appropriate dilutions of homogenates were made, and measured drops were placed on the surface of solid medium. After incubation, colony counts were made and the total population of tubercle bacilli, expressed as culturable units per ml. of tissue, was calculated.

RESULTS

In tables 1 and 2 may be seen the results of tests of the *in vitro* activity of pyrazinamide on strain H37Rv and on the "resistant" strain E11L derived from it. As may be seen in table 1, strain H37Rv was resistant to more than 400 γ of pyrazinamide per ml. at pH 7.0. It was inhibited, however, by 6.2 γ and by 25 γ of pyrazinamide per ml. at pH 5.0 and 5.5, respectively. The data in table 2 indicate that, in a similar experiment, strain E11L was not inhibited by pyrazinamide even at pH 5.0 to 5.5. Strain E11L still maintained its original susceptibility to isoniazid concentrations of 0.03 γ per ml.

In figures 1 and 2 may be seen the characteristic curves of the populations of tubercle bacilli in the lungs and spleens of mice which were infected with strain H37Rv and which had remained untreated or had been given pyrazinamide or isoniazid. As seen in figure 1, pyrazinamide effected a marked reduction in the

TABLE 1

ACTIVITY OF PYRAZINAMIDE ON M. TUBERCULOSIS STRAIN H37Rv WITH VARIATION OF ENVIRONMENTAL ACIDITY

pH	Drug (γ per ml.)									
	400	200	100	50	25	12.5	6.2	3.1	1.6	0
5.0	0	0	0	0	0	0	0	1+	1+	1+
5.5	0	0	0	0	0	2+	2+	3+	3+	3+
6.0	2+	2+	4+	4+	4+	4+	4+	4+	4+	4+
6.5	2+	2+	4+	4+	4+	4+	4+	4+	4+	4+
7.0	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

TABLE 2

ACTIVITY OF PYRAZINAMIDE AGAINST THE "RESISTANT" STRAIN OF M. TUBERCULOSIS, STRAIN E11L, WITH VARIATIONS IN ENVIRONMENTAL ACIDITY

pH	Drug (γ per ml.)									
	400	200	100	50	25	12.5	6.2	3.1	1.6	0
5.0	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
5.5	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
6.0	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
6.5	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
7.0	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

total population of tubercle bacilli in the lungs. At three weeks and at eight weeks after the start of pyrazinamide therapy, the populations of tubercle bacilli were so low that in 5 of 6 animals they were below the detection limits of the methods employed. After twelve weeks of pyrazinamide therapy, however, in 2 of 3 animals, the populations of tubercle bacilli were again at a relatively high level. It was from one such animal that strain E11L was isolated. For comparison, the effects of isoniazid and of pyrazinamide and isoniazid together are also depicted. As may be seen (figure 1) in the lung, these two therapies also effected a marked reduction in the total populations of tubercle bacilli, so that the microbial census was below the limits of detection in some animals after three weeks of therapy.

In figure 2 are shown the effects of these drugs on the populations of tubercle bacilli in the spleens of animals infected with strain H37Rv. In the control animals the populations of tubercle bacilli displayed the usual rise followed by a fall with stabilization thereafter. Both pyrazinamide and isoniazid given alone effected a marked reduction in the number of tubercle bacilli in the spleen, the latter drug being more potent in this instance. The administration of pyrazinamide and isoniazid together caused a marked fall in the populations of tubercle bacilli with the eventual apparent disappearance of all tubercle bacilli from the spleen, as has been discussed in detail elsewhere (9–11).

In sharp contrast to the above observations were the effects of these drugs on the E11L strain of tubercle bacilli. In figure 3 it may be seen that the behavior of the populations of tubercle bacilli in the lungs of control animals and in those given pyrazinamide was almost exactly the same. The effectiveness of isoniazid against the E11L strain was the same as the effectiveness of this drug in animals infected with the parent strain H37Rv. The effects of pyrazinamide and isoniazid given together to animals infected with the E11L strain of tubercle bacilli were the same as when the isoniazid was given alone.

In figure 4 may be seen the populations of tubercle bacilli in the spleens of animals infected with strain E11L. In the animals treated with pyrazinamide the populations of tubercle bacilli were maintained at a slightly higher level than in the control animals. The usual effects of isoniazid when given singly were seen, and the effects of the administration of pyrazinamide and isoniazid together were the same as when isoniazid was given alone.

In other experiments in this laboratory, two additional strains of tubercle bacilli resistant to pyrazinamide in an acidic environment *in vitro* and apparently resistant to the drug *in vivo* have been isolated from pyrazinamide-treated mice infected with the H37Rv strain. Neither of these two strains was subjected to further study. It is probable that pyrazinamide-resistant derivatives of H37Rv can be obtained relatively frequently from mice treated with pyrazinamide alone. For, in the total experience in this laboratory, the apparent resurgence of the populations of tubercle bacilli in the mouse tissues after an initial pyrazinamide-

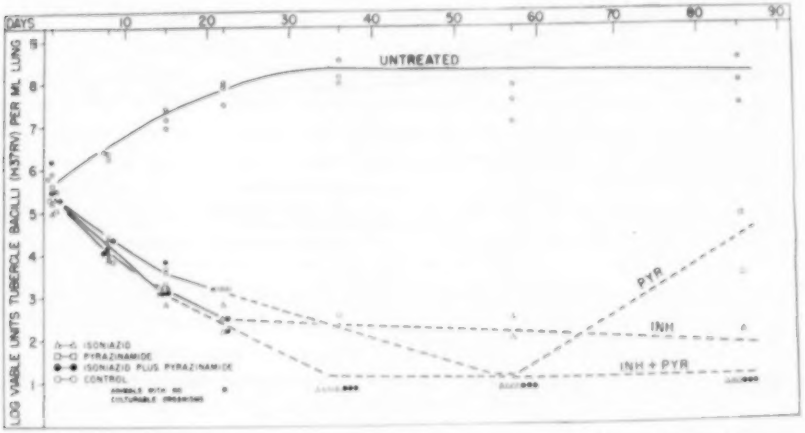

FIG. 1. The effect of pyrazinamide and isoniazid given singly and together on the population of *M. tuberculosis* in the mouse lung. The infecting strain of tubercle bacilli, H37Rv, was susceptible to pyrazinamide.

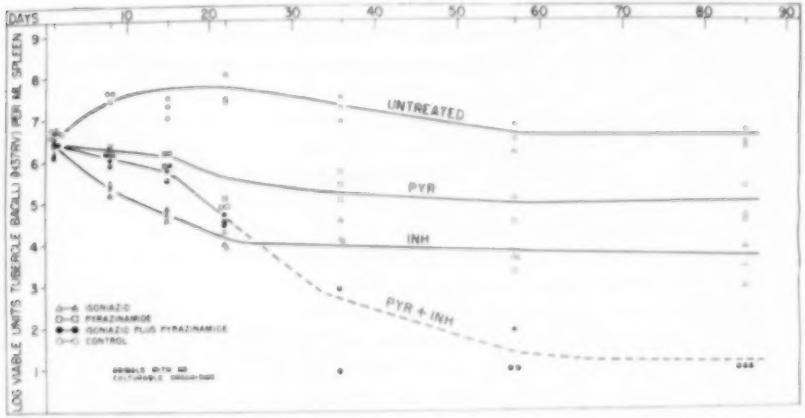

FIG. 2. The effect of pyrazinamide and isoniazid given singly and together on the population of *M. tuberculosis* in the mouse spleen. The infecting strain of tubercle bacilli, H37Rv, was susceptible to pyrazinamide.

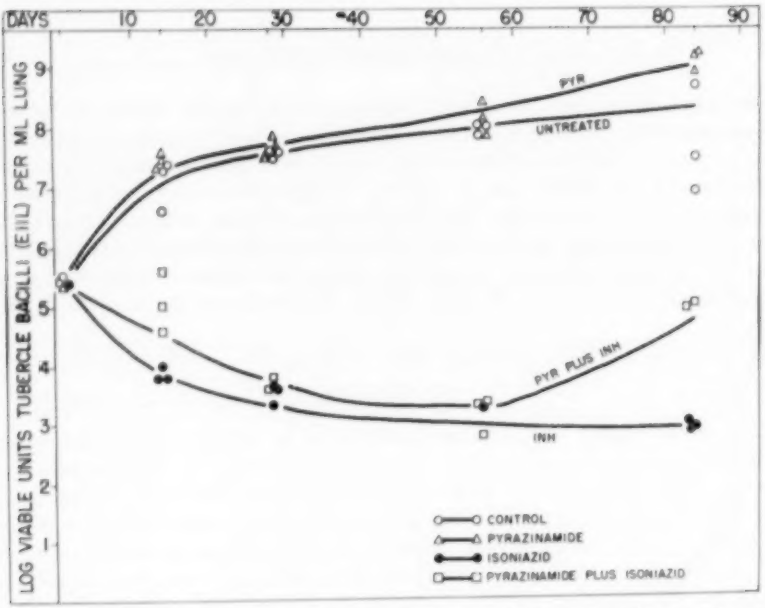

FIG. 3. The effect of pyrazinamide and isoniazid given singly and together on the population of *M. tuberculosis* in the mouse lung. The infecting strain of tubercle bacilli used here, E111L, proved resistant to pyrazinamide.

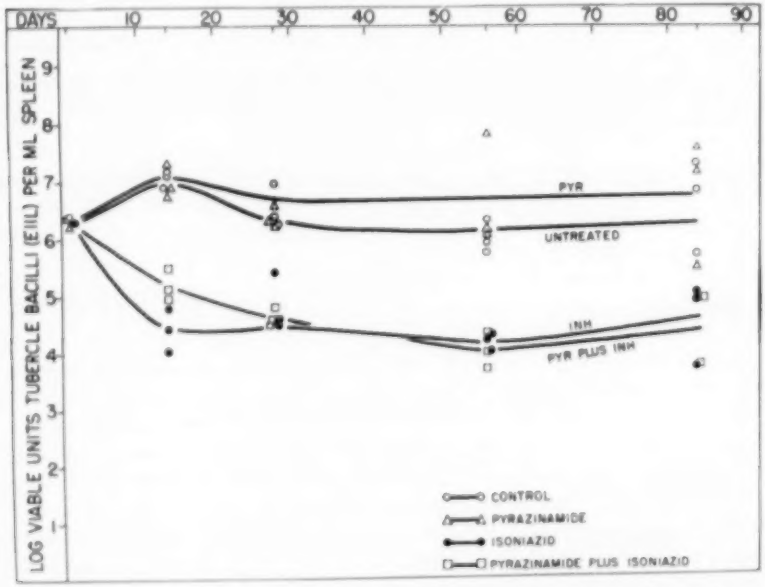

FIG. 4. The effect of pyrazinamide and isoniazid given singly and together on the population of *M. tuberculosis* in the mouse spleen. The infecting strain of tubercle bacilli used here, E111L, proved resistant to pyrazinamide.

induced fall was observed not infrequently. No attempt has been made, however, to test all such strains for pyrazinamide susceptibility in an acidic environment *in vitro*.

It is apparent from the experiments described that the pyrazinamide-resistant strain E11L is pathogenic for mice, for this strain of tubercle bacilli showed the same capacity to multiply in the tissues of the mice as displayed by the parent strain H37Rv. As will be described in detail elsewhere (8), the E11L strain is likewise fully pathogenic for guinea pigs.

DISCUSSION

It is generally agreed that pyrazinamide is a potent antituberculous drug in experimental animals and in man. Thus the absence of *in vitro* activity against tubercle bacilli under customary methods of testing is paradoxical, especially in view of the lack of evidence that the drug can be broken down *in vivo* to compounds which possess any *in vitro* activity. Consequently the observation that pyrazinamide inhibited tubercle bacilli of human origin at low pH (6) appeared to have added significance. The present work adds further evidence that the activity of pyrazinamide on tubercle bacilli at a low pH may be correlated with its *in vivo* activity.

The total evidence thus far is in three parts: (*1*) Tubercle bacilli of human origin (H37Rv) are inhibited by pyrazinamide at low pH and the drug is active on these microorganisms *in vivo*. (*2*) Tubercle bacilli of bovine origin are not inhibited by pyrazinamide at low pH and the drug is inactive on them *in vivo*. (*3*) The evidence from the present study shows that a strain of tubercle bacilli (E11L) derived from the "pyrazinamide-susceptible" strain H37Rv was resistant to pyrazinamide *in vivo* and was likewise resistant to the drug when tested in an acidic environment *in vitro*.

Environments comparable in acidity with those necessary for the action of pyrazinamide on tubercle bacilli *in vitro* have been shown to be present within monocytes (12, 13) and appear to exist within inflammatory-necrotic areas *in vivo* (14). Moreover, Mackaness has shown that pyrazinamide is active against tubercle bacilli of human origin when they are situated within monocytes (15). He states that the pH of the extracellular environment was maintained within the physiologic range throughout the experimental period.

It should be emphasized, however, that the demonstration of a correlation between the pyrazinamide susceptibility in an acidic environment *in vitro* and susceptibility to the drug *in vivo* does not necessarily imply that an acidic environment is an essential prerequisite for pyrazinamide action in the tissues. Indeed, in other studies (10) evidence is presented which suggests that it is the capacity of a tubercle bacillus to undergo some alteration of a specific type that determines its susceptibility to pyrazinamide. The environmental influences which have been shown to facilitate this pyrazinamide-tubercle bacillus reaction *in vitro* (presumably by inducing an alteration in the bacillus) are the acidic environment and the intracellular location of the parasite. The influence demonstrated to be operative *in vivo* is the concurrent exposure to another antituberculous drug.

Whether these various environmental influences exert their effects on the tubercle bacilli (or the drug-parasite reaction) through some common factor such as acidity, or whether an acid environment represents just one of several types of influence capable of enhancing the reaction, cannot be stated at present. Environmental acidity as a factor common to these environmental influences of demonstrated effectiveness is an attractive notion for speculation. This is particularly true in view of the observations of Ishimori (16) that a strain of tubercle bacilli of bovine origin was better adapted for optimal growth in an acidic environment than was the case with tubercle bacilli of human origin. Nevertheless, with the presently available evidence, it is not possible to conclude that a single common factor, such as the acidity of the environment, exists in the production of the inhibition of tubercle bacilli by pyrazinamide. In any event, the end result of the operation of these various influences on this drug-parasite reaction appears to be uniquely specific for pyrazinamide and for strains of tubercle bacilli of human origin.

In the present and previous studies (9, 10) of mice infected with the H37Rv strain of tubercle bacilli and treated with pyrazinamide, the emergence of derivative strains resistant to pyrazinamide has not been noted during the early weeks of therapy. On the contrary, the populations of tubercle bacilli in the lungs uniformly fall to a low level during the early weeks of pyrazinamide administration and the populations in the spleen either fall or show no increase. The appearance of strains of tubercle bacilli capable of maintaining a high census in the lung despite pyrazinamide therapy, such as was the case with the E11L strain, has been observed only after six or eight weeks of therapy and only in experimental groups in which the preceding lung population of tubercle bacilli had been low.

The late appearance of the pyrazinamide-resistant strains and their apparent emergence from the minority which survived the initial impact of the pyrazinamide therapy suggest that the pyrazinamide-resistant cells were present in the original inoculum of tubercle bacilli. The observations thus suggest that the H37Rv strain of tubercle bacilli represents a mixture in terms of pyrazinamide susceptibility and that a minority of the cells in the mixture are identical with bovine tubercle bacilli with respect to their inability to become susceptible to pyrazinamide. Attempts are being made to subject this hypothesis to experiment by the study of bovine and human strains of tubercle bacilli.

It should be mentioned that the time interval between the start of pyrazinamide therapy and the detection of pyrazinamide-resistant strains of tubercle bacilli in mice is about the same as the interval between the onset of defervescence and the first appearance of the relapse which is seen not infrequently in human infections. Yeager and his associates in their original study of the use of pyrazinamide alone found this interval to be approximately six weeks in the patients whose infections relapsed during therapy (2). An interval of this length would be compatible with the hypothesis that a small number of tubercle bacilli without the capacity to become susceptible to pyrazinamide was present before treatment and steadily emerged to predominance thereafter.

SUMMARY

In the course of a large number of experiments involving the pyrazinamide treatment of mice infected with the H37Rv strain of human tubercle bacilli, the appearance during therapy of strains of tubercle bacilli apparently resistant to the influence of pyrazinamide has been observed not infrequently. One such strain, designated E11L, has been studied in detail both *in vitro* and *in vivo*. It was found that the E11L strain maintained full pathogenicity for mice as determined by its ability to multiply in this host to the same extent as the parent strain H37Rv. Unlike the parent strain, however, the E11L strain of tubercle bacilli was not inhibited by pyrazinamide *in vitro* at pH 5.0 to 5.5. This pyrazinamide resistance *in vitro* was correlated with pyrazinamide resistance *in vivo*. The E11L strain in experimentally infected mice was completely uninfluenced by intensive therapy with pyrazinamide in contrast to the parent H37Rv strain which was highly susceptible to the drug when tested under similar conditions.

The observations suggest that the H37Rv strain of human tubercle bacilli represents a mixture which includes a small number of cells resembling bovine tubercle bacilli in their lack of capacity to become susceptible to pyrazinamide *in vivo* or in an acidic environment *in vitro*.

SUMARIO

Resistencia de los Bacilos Tuberculosos a la Piracinamida In Vitro

En el transcurso de un gran número de experimentos que comprendieron el tratamiento con piracinamida de ratones infectados con la cepa H37Rv de bacilos tuberculosos humanos, se observó con cierta frecuencia la aparición durante la terapéutica de cepas de bacilos tuberculosos aparentemente resistentes al influjo de la piracinamida. Una de esas cepas, denominada E11L, ha sido estudiada a fondo, tanto *in vitro* como *in vivo*. Notóse que la cepa E11L mantenía plena patogenicidad para los ratones, determinado esto por su capacidad para multiplicarse en este huésped en el mismo grado que la cepa matriz H37Rv. No obstante, en contraposición a la cepa matriz, la cepa E11L del bacilo tuberculoso no fué inhibida por la piracinamida *in vitro* a un pH de 5.0 a 5.5. La piracinamido-resistencia *in vitro* se correlacionó con la piracinamido resistencia *in vivo*. La cepa E11L en los ratones infectados experimentalmente no fué afectada en lo más mínimo por la terapéutica intensa con piracinamida en contraposición a la cepa matriz H37Rv que fué sumamente susceptible a la droga cuando fué comprobada en condiciones semejantes.

Estas observaciones sugieren que la cepa H37Rv de bacilos tuberculosos humanos representa una mezcla que comprende un pequeño número de células parecidas a bacilos tuberculosos bovinos en su falta de capacidad para susceptibilizarse a la piracinamida *in vivo* o en medio acidógeno *in vitro*.

RESUME

Résistance des bacilles tuberculeux à la pyrazinamide in vitro

Au cours de nombreuses expérimentations du traitement par la pyrazinamide de souris infectées par la souche de bacille tuberculeux humain H37Rv, l'apparition de bacilles paraissant résistants à l'action de la pyrazinamide n'a pas été exceptionnelle. Une de ces souches, dénommée E11L a été étudiée en détail *in vitro* et *in vivo*. Il a été découvert que la souche E11L conservait une entière pathogénicité pour la souris, comme le démontre son aptitude à se reproduire dans l'hôte, autant que la souche H37Rv. Toutefois, à l'encontre

de la souche mère, la souche de bacille tuberculeux E11L n'était pas inhibée *in vitro* par la pyrazinamide au pH 5-5,5. Chez la souris infectée expérimentalement, la souche E11L n'était absolument pas influencée par un traitement intensif avec la pyrazinamide, contrairement à la souche mère H37Rv, qui était très sensible à cet agent dans les tests pratiqués dans les mêmes conditions expérimentales.

Les observations suggèrent que la souche de bacilles tuberculeux humains H37Rv représente un mélange comprenant un petit nombre de cellules ressemblant au bacille tuberculeux bovin par leur défaut d'aptitude à acquérir une sensibilité à la pyrazinamide *in vitro* ou dans un milieu acide *in vitro*.

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COMPLICATIONS ASSOCIATED WITH POLYVINYL-FORMAL SPONGE PROSTHESIS AND PLOMBAGE IN THE SURGICAL MANAGEMENT OF PULMONARY DISEASES[1]

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INTRODUCTION

From time to time, thoracic surgeons have made enthusiastic use of foreign materials as compressive devices or as prostheses in an effort to avoid deformity of the thorax resulting from thoracoplasty or to prevent overexpansion of the portion of the lung remaining after pulmonary resection.

In many parts of the United States, interest in resection as part of the management of pulmonary tuberculosis has waxed, while enthusiasm for permanent collapse procedures has waned. Distention of the portion of the lung remaining after pulmonary resection has proved less deleterious than was once supposed. Today there is little need for tailoring the thoracic wall to fit residual pulmonary tissue, which will expand and fill the pleural cavity.

The experimental work of Grindlay and associates (1, 2) prompted the present writers to use polyvinyl-formal plastic (Ivalon®) sponge in the surgical management of a small group of patients with tuberculosis or carcinoma of the lung. The purpose of this paper is to call attention to the complications observed following use of the sponge as a prosthesis in the pleural space and to report the absence of complications related to the use of this material in the extrapleural space as a substitute for compressive thoracoplasty.

MATERIAL AND PROCEDURE

The experience of the writers with polyvinyl-formal plastic sponge in the surgical treatment of pulmonary diseases is represented by 30 patients. Of these, 19 were carrying the sponge as a prosthesis after pulmonary resection and 11 were carrying it in the extrapleural space as a substitute for compressive thoracoplasty.

Pulmonary Resection with Plastic-Sponge Prosthesis

The 19 resections in this series were performed between October 18, 1949, and October 15, 1953. A contoured piece of polyvinyl-formal sponge was placed in the pleural space and sutured to the thoracic wall in two or three places. Care was taken not to crowd the sponge into the space. In some instances a large flap of parietal pleura was laid over the raw surface of the lung so that, in effect, the sponge lay in an extrapleural position. Closed intrapleural drainage was maintained through two or three rubber catheters for several days after operation. With one exception, the sponge was inserted at the time of resection. The sponge was placed in the thorax of this patient six years after pneumonectomy.

[1] Mineral Springs Sanatorium, Cannon Falls, Minnesota; Section of Surgery, Mayo Clinic and Mayo Foundation, Rochester, Minnesota. The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

Extrapleural Pneumonolysis with Plastic-Sponge Plombage

Eleven patients in this series had 13 extrapleural pneumonolyses performed between September 12, 1951, and October 29, 1954, 2 of the patients having bilateral procedures. An extrapleural cleavage plane was established and carried down to the level of the fourth rib anteriorly, to the sixth rib posteriorly, and to the root of the lung medially. A contoured piece of polyvinyl-formal sponge was placed so that it lay loosely in the prepared extrapleural space. Closed intrapleural drainage was maintained through a single rubber catheter for a few days if the pleura had been opened accidentally during operation.

OBSERVATIONS

Complications related to the sponge developed in 9 patients. These complications consisted of the postoperative accumulation of fluid or air, or both, in the region of the sponge. Certain features in each case have been examined in an effort to find some clinical differences between the cases in which complications developed and those in which they did not develop. The salient points in each case are given in figure 1.

The complications which have been encountered thus far have been limited to patients in whom the sponge has been used as a prosthesis following pulmonary resection. No complications have appeared among those patients carrying the sponge in the extrapleural space as a substitute for compressive thoracoplasty.

The onset of complications in this series was often heralded by productive cough. The chief roentgenographic signs were those of aeration or of effusion in the region of the sponge. Bronchopleural fistulas communicating with the space occupied by the sponge were associated with either one of these signs. The fistulas varied from patient to patient. They could be large, small, single, or multiple. The time between resection and the appearance of complications varied widely. In one patient, signs of aeration were present from the time of operation, while in another the signs of effusion did not appear for thirty-eight months. In only 3 cases did signs of trouble appear within the first year after operation.

In 4 cases, purulent material was observed in the region of the sponges when these were removed. Cultures of the material were made in 2 cases. In one of these cases the cultures were positive for tubercle bacilli but in the other they were negative for tubercle bacilli, Brucella, or fungi.

Resection was performed on the left side in 13 patients and on the right in 6. Complications were limited to those who underwent resection on the left. A tuberculous cavity was opened inadvertently during resection in 2 patients. One patient developed signs of effusion and the other developed signs of aeration in the region of the sponge. Removal of the sponge was required subsequently in both patients.

Sixteen patients underwent resection for tuberculosis of the lung. Complications developed in 8 of these patients, and all 8 had *M. tuberculosis* in the sputum within ten weeks prior to operation. On the other hand, only 2 of the 8 patients who remained free of complications had tubercle bacilli in the sputum prior to operation. Six of the 11 patients having extrapleural pneumonolysis had tubercle bacilli in the sputum prior to operation.

Lung and sponge were partially separated by a flap of pleura in 9 of the 16

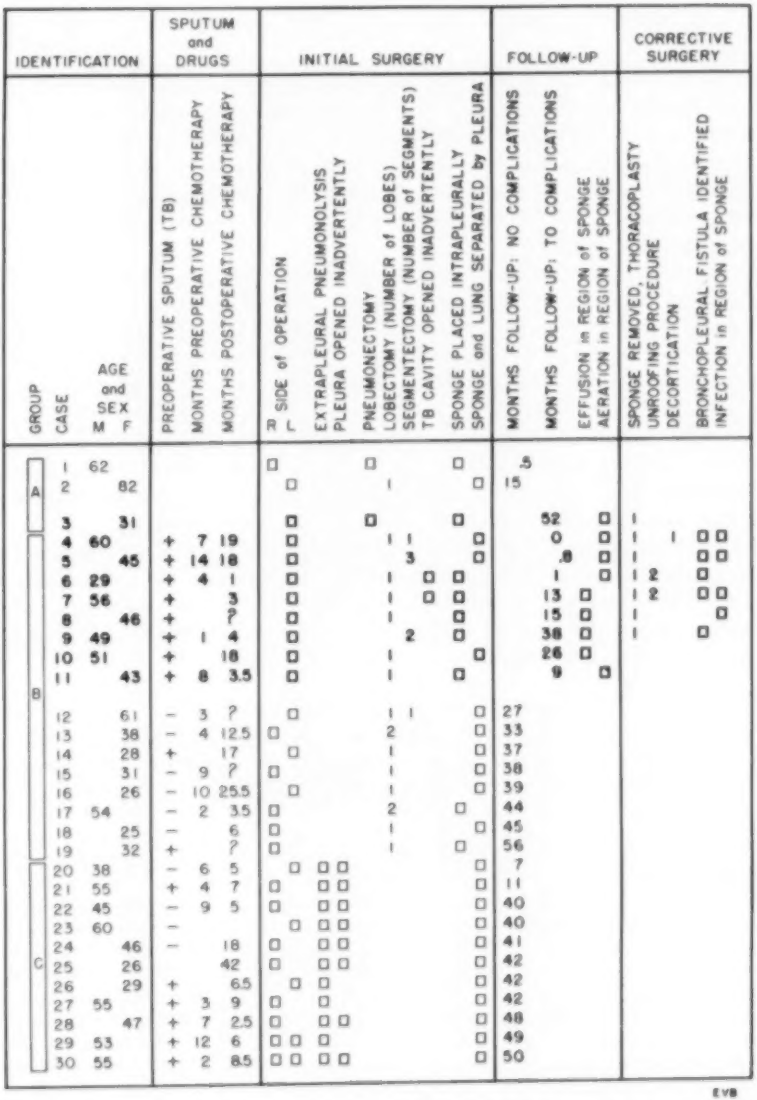

FIG. 1. Selected data from 30 patients having plastic-sponge prosthesis and plombage. *Group A* includes patients who underwent resection for carcinoma of the lung. *Group B* includes those who underwent resection for tuberculosis of the lung. *Group C* includes those who underwent extrapleural pneumonolysis for compression of tuberculous pulmonary tissue. Boldface characters represent patients in whom complications developed after operation. "Preoperative sputum" refers to the status of the sputum with regard to tubercle bacilli within ten weeks of the initial operation. "Preoperative chemotherapy" refers to antituberculous drug therapy given immediately prior to the initial operation. Numerals under "corrective surgery" refer to first and second operations.

patients who had resections for tuberculosis of the lung. Complications developed in only 3 of these. On the other hand, complications appeared in 5 of the 7 patients who had pulmonary resection with placement of the sponge in direct contact with the lung. No trouble was encountered among 11 patients in whom the sponge was used as a substitute for compressive thoracoplasty and was completely separated from the underlying lung by its position in a surgically prepared extrapleural space.

Three patients died of causes unrelated to the presence of the sponge. The first patient (*Case 1*) died thirteen days after radical pneumonectomy for carcinoma of the lung. The second (*Case 2*) died of metastasis fifteen months after lobectomy for carcinoma of the lung. The third patient (*Case 21*) died of heart disease eleven months after extrapleural pneumonolysis for permanent compression of cavitary tuberculosis.

Corrective operations: Seven of the 9 patients with complications have required corrective surgical treatment. In each case this has consisted of thoracotomy with removal of the sponge and thoracoplasty. In 2 cases a subsequent procedure was done to unroof and close a space on the side of thoracoplasty.

Two patients with complications have not required corrective surgical treatment because the signs of complications in each case have been transient. In one, signs of aeration appeared in the region of the sponge nine months after resection. In the other, signs of effusion appeared in the region of the sponge twenty-six months after resection.

REPORT OF ILLUSTRATIVE CASES

Case 8. A 46-year-old white woman was admitted to Mineral Springs Sanatorium for the first time on April 23, 1952, with far advanced pulmonary tuberculosis. There was moderately extensive involvement of both lungs, with cavitation in the upper portion of the left. Culture of sputum obtained by gastric lavage was positive for *M. tuberculosis*. Bronchoscopic examination revealed stenosis of the left upper lobe bronchus.

Treatment with dihydrostreptomycin given semiweekly and para-aminosalicylic acid sodium (PAS) given daily was instituted early in May. Because of intolerance, the use of sodium PAS was discontinued and the daily administration of isoniazid was started after one year of treatment. Transient tuberculous pleurisy with effusion developed in June, 1952. Thereafter, moderate resolution of the pulmonary lesions occurred. Cultures of material obtained by gastric lavage became negative for tubercle bacilli in August, 1952, and remained negative except for a single positive result one month prior to operation.

The anterior and apical-posterior segments of the left upper lobe and the superior segment of the left lower lobe were excised on June 17, 1953. The bronchial stumps were pleuralized and a flap of pleura was dissected from the thoracic wall and sutured over the raw surface of the remaining lung. A small contoured polyvinyl-formal plastic sponge was placed in the apex of the pleural space so that it was separated from the lung by the pleural flap. The postoperative course was uneventful.

Signs of aeration in the region of the sponge appeared three weeks after operation (figure 2*a*), but gradually disappeared during the ensuing months (figure 2*b*). Late in January, 1954, the patient became ill with cough and malaise. Signs of effusion and a rim of air about the sponge appeared in her next roentgenogram in March, 1954. Because of the facts that her symptoms had disappeared, the gastric cultures contained no tubercle bacilli, and the air around the sponge had decreased, it was decided to defer surgical intervention but to extend chemotherapy through November, 1954.

FIG. 2. Postero-anterior views of thorax in *Case 8*. *a*. Early postoperative view showing aeration in region of sponge in left upper part of thorax. *b*. Satisfactory appearance of sponge four months later. *c*. Effusion in region of sponge with surrounding rim of air present prior to removal of sponge two and one fourth years after insertion.

FIG. 3. Postero-anterior views of thorax in *Case 11*. *a*, Satisfactory appearance of sponge in left upper part of thorax four months after insertion. *b*, Aeration present in region of sponge five months later. *c*, Air has reabsorbed and appearance is satisfactory two and three-fourths years later.

Late in January, 1955, the patient became ill with cough and malaise but she delayed returning for examination. Signs of effusion and a rim of air about the sponge appeared in her next roentgenogram in May, 1955 (figure 2*c*). She was readmitted to the sanatorium on June 17. Numerous gastric cultures were negative for tubercle bacilli. Bronchoscopic examination shortly after admission revealed copious purulent secretion coming from the stump of the superior segment of the left lower lobe bronchus. The left upper lobe bronchus was slightly narrowed, but the stumps of the resected segments were not visualized. Chemotherapy was reinstituted, isoniazid being given daily and streptomycin injected semiweekly. The administration of streptomycin was stopped three weeks later because of intolerance, and calcium PAS was substituted.

Thoracotomy was performed on September 21, 1955. Considerable thick purulent fluid surrounded the sponge; the fluid was not cultured. Multiple, small, bronchopleural fistulas made closure by suture difficult. Therefore, the fistulas were covered with adjacent muscle. A six-rib thoracoplasty was done.

The patient was dismissed on November 9, 1955, with the advice to continue chemotherapy for several months. The roentgenographic appearance of the thorax in February, 1956, was satisfactory.

This case typifies the course of patients who required further surgical treatment because of complications from the sponge. Bronchopleural fistulas were demonstrated at the time of operation, as they were in all but 2 of the 7 patients who subsequently underwent operation because of the development of complications.

Case 11. A 43-year-old white woman was admitted to Mineral Springs Sanatorium on February 3, 1951, with moderately advanced pulmonary tuberculosis confined largely to the upper portion of the left lung. Her sputum was positive for *M. tuberculosis* by direct stain and by culture. Pneumoperitoneum was induced and reinforced by left phrenemphraxis in February. Semiweekly administration of dihydrostreptomycin and daily administration of PAS were started at the same time. Bronchoscopic examination on March 19 disclosed pronounced stenosis and active tuberculous ulceration of the left upper lobe bronchus. Re-examination on November 15, 1951, showed healing of the active endobronchial disease, but the bronchial stenosis persisted. Pneumoperitoneum was discontinued prior to resection. Gastric cultures remained positive for *M. tuberculosis* up to the time of operation.

The left upper lobe was removed on November 21, 1951. A contoured piece of polyvinylformal plastic sponge was inserted in the space left by removal of the lobe, in direct contact with the remaining portion of the lung, and sutured to the thoracic wall.

Chemotherapy was maintained for three and one half months after operation. Gastric cultures for *M. tuberculosis* were negative during this period. In March, 1952, the patient became hoarse and noticed a parasternal wheeze. Culture of sputum obtained by gastric lavage again yielded tubercle bacilli. Bronchoscopic examination on May 14, 1952, showed thick purulent exudate in the left main bronchus and reddening of the mucosa, but the stump of the left upper lobe bronchus appeared normal. Chemotherapy was instituted and continued throughout the balance of her stay in the sanatorium. Gastric cultures last yielded tubercle bacilli in June, 1952. The roentgenographic appearance of the thorax at the end of June was satisfactory (figure 3*a*).

Signs of aeration appeared in the region of the sponge nine months after operation (figure 3*b*), but there were no accompanying symptoms. The patient was dismissed from the sanatorium on December 21, 1952. She moved promptly to another state and no longer remained under our observation. Aeration in the region of the sponge was not evident in a follow-up roentgenogram submitted for review in August, 1955 (figure 3*c*).

This patient is one of 2 who have demonstrated roentgenographic changes in the region of the sponge and who have not required reoperation. Aeration of

the region of the sponge in this patient was not accompanied by symptoms and it later cleared.

DISCUSSION

In 1952 Gale and associates (3) reported their observations on the use of polyvinyl-formal plastic-sponge prosthesis following resection in 50 patients. At the time of their report, 46 patients were free of complications; 8 of them had been followed for one year, 26 for six to twelve months, and 12 for less than six months. They encountered difficulties in the remaining 4 patients. One died of contralateral spontaneous pneumothorax eighteen hours after operation and another died of a transfusion reaction six days after operation. In the other 2 patients, infection developed around their sponges, and this required removal of the sponge twelve days after operation in one patient and several weeks after operation in the other. More recently, Kukral and associates (4) reported that complications occurred in 8.9 per cent of the patients in whom a polyvinyl-formal sponge had been inserted into the extrapleural space. They considered these complications to be of an allergic nature.

A review of the cases reported here emphasizes that patients carrying a polyvinyl-formal plastic sponge as a prosthesis after pulmonary resection have a prognosis that is different from that of patients carrying this material in the extrapleural space as a substitute for thoracoplasty. The writers' experience with the use of such a sponge as a physical replacement for excised pulmonary tissue has been disappointing. On the other hand, observation of a limited number of cases suggests that a sponge can be used dependably in the extrapleural space for permanent compression of the underlying lung in the collapse therapy of pulmonary tuberculosis.

It is believed that the occurrence of complications in 9 of 19 patients who underwent resection in this series represents an excessively high incidence when compared with the incidence of postoperative difficulties reported in tuberculous patients following pulmonary resection (5).

SUMMARY

Polyvinyl-formal plastic sponge was used in 19 patients as a prosthesis following pulmonary resection. Complications occurred in 9 patients and required removal of the sponge in 7. No complications occurred in 11 patients who had this material put in the extrapleural space as a substitute for compressive thoracoplasty.

SUMARIO

Complicaciones Enlazadas con la Prótesis y el Plumbaje con Compresa Polivinilo-Formálica en la Asistencia Quirúrgica de las Afecciones Pulmonares

A continuación de la resección pulmonar, en 19 enfermos se usó como prótesis una compresa plástica de polivinilo-formal. En 9 enfermos hubo complicaciones que exigieron el retiro de la compresa en 7. No aparecieron complicaciones en 11 sujetos en quienes se colocó este material en el espacio extrapleural como substituto de la toracoplastia compresiva.

RESUME

Complications associées à la prothèse et au plombage avec une éponge Polyvinyl-Formal dans le traitement chirurgical d'affections pulmonaires

L'éponge plastique Polyvinyl-Formal a été utilisée comme materiel de prothèse après résection pulmonaire chez 19 malades. Des complications sont survenues dans 9 cas et chez 7 d'entre eux l'éponge a du être enlevée. Aucune complication n'est survenue chez 11 malades pour lesquels cette matière avait été placée dans la cavité extrapluerale en remplacement d'une thoracoplastie compressive.

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THE LOS ANGELES COUNTY JAIL CHEST ROENTGENOGRAPHIC SCREENING PROGRAM[1, 2]

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INTRODUCTION

It has been demonstrated repeatedly that the tuberculosis rate among prisoners is significantly higher than in the general population. Bettag (1) reported, in a survey at a state prison in Illinois, a prevalence of 8.9 active cases per thousand persons, which he considered to be seven times the rate of Illinois as a whole. Garry (2) surveyed penal and correctional institutions in Ohio and found suspected tuberculosis in 3.3 per cent of inmates and 2.3 per cent of employees, the former three times and the latter twice the general population rate. Katz and Plunkett (3) reported a prevalence rate of 12 per thousand in males and 7 per thousand in females in all the institutions of the New York State Department of Correction as compared with an estimated prevalence rate of 3 per thousand in the general population.

Despite these facts, it appears from the paucity of reports that there are very few continuous case-finding programs in prisons, and most of these are confined to federal and state penitentiaries. Beatty and Horst (4), in 1949, sent questionnaires to penal institutions throughout the United States and Canada. Answers received from fifty-five state and seventeen federal institutions in the United States, and six Canadian institutions, indicated that only a few had continuous case-finding programs. Physical examination was the most commonly employed diagnostic procedure, large chest films being taken when indicated by physical examination. Seventeen institutions, most commonly United States federal, used small roentgenograms at irregular intervals and usually under the auspices of an outside agency.

In mid-1954, under the joint sponsorship of the Los Angeles County Sheriff's Department and the Los Angeles County Tuberculosis and Health Association, a new program for tuberculosis control was instituted at the Los Angeles County Jail. A combination 70 mm. photofluorographic and 14- by 17-inch roentgenographic unit and darkroom facility was installed at the county jail. In conjunction with this a "minimal security" tuberculosis prison hospital, the Mira Loma facility, with a capacity of approximately one hundred beds, was opened.

PLAN OF INVESTIGATION

The details of the physical arrangements and procedures will vary with individual programs and requirements. Only those which may be of general interest will be described. The X-ray unit is located in the identification laboratory of the jail. As soon as the prisoner

[1] Presented, in part, before the Community Action Session at the annual meeting of the National Tuberculosis Association, New York City, New York, May 23, 1956.

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has his identification photographs taken, a 70 mm. photofluorogram is obtained as a part of the regular booking procedure. During the night the films are developed, and in the morning they are interpreted by a radiologist. The tuberculosis control officer of the jail is informed of urgent cases immediately so that they may be segregated as soon as possible. Appropriate 14- by 17-inch views are taken in each instance in which tuberculosis or other significant disease is suspected. Prisoners suspected of having tuberculosis are segregated in separate rooms in the jail, and appropriate studies are instituted by the jail physicians and their consultant in chest diseases. These studies include physical examination, sputum studies, and gastric washings. When a diagnosis of active tuberculosis is made, the prisoner is sent to the Mira Loma facility. In certain cases requiring higher security measures, a prisoner may be kept segregated at the county jail.

Of the 5,000 to 5,250 prisoners in the custody of the Los Angeles County Sheriff, approximately 2,500 to 3,000 are in the county jail itself while the remainder are in six road camps, the Terminal Island facility for women, and the Honor Ranch. It may be seen that segregation of tuberculous prisoners offers something of a problem when it is added to the other types of segregation employed: there is segregation according to types of crime as well as segregation of first offenders and recidivists, morals offenders, and narcotic addicts and peddlers. The average stay of a prisoner in the Los Angeles County Jail is five to six months. A prisoner who still has active tuberculosis at the time of release from prison is referred to the county health department which in turn notifies local authorities so that proper disposition can be made.

OBSERVATIONS

From July 1, 1954, to June 30, 1955, 47,072 miniature films were made. Of these, 5,730 were re-examinations. A total of 41,342 persons, or 99.43 per cent of prisoners booked at the county jail, were examined during this period (table 1). There were 1,289 tuberculosis suspects found in this group, of whom 604 persons, or 14.6 per thousand, were subsequently diagnosed as having tuberculosis, both active and inactive (table 2). In 270 persons tuberculosis was excluded, and in the remaining 415 prisoners the diagnosis has not been completed. To date the diagnosis has been completed in 68 per cent of the tuberculosis suspects.

TABLE 1

NUMBER OF EXAMINATIONS AND TUBERCULOSIS SUSPECTS

	Number	Per Cent
Total prisoners booked	47,340	100.00
Number of examinations	47,072	99.43
Persons examined	41,342	100.00
Tuberculosis suspects	1,289	3.12

TABLE 2

FINAL DIAGNOSIS IN TUBERCULOSIS SUSPECTS

Final Diagnosis	Number	Diagnosis Completed
Tuberculosis	604*	68%
Not tuberculosis	270	
Not complete	415	
	1,289	

* 14.6 per 1,000 persons

TABLE 3

CASES OF ACTIVE TUBERCULOSIS

		Rate*
Total cases of active tuberculosis	260	6.3
Violation of isolation order	143	3.5
Cases unknown to jail	117	2.8
Known to health department	33	0.7
Previously unknown	84	2.1

* Per 1,000 persons

The total number of cases of active tuberculosis was 260, or 6.3 per thousand persons (table 3). More than one-half of these, 143, were persons with known cases of tuberculosis, as they had been imprisoned specifically for violation of an isolation order.

California law provides that the local health officer must issue a quarantine and isolation order when he determines it is necessary for the preservation of the public health to segregate any person with infectious tuberculosis (5). Violation of this order is a misdemeanor and, upon conviction by a local court, the violator may be confined for a period up to twelve months.

There were 117 prisoners with active tuberculosis whose disease had not been known at the time of their confinement, representing a rate of 2.8 per thousand. Subsequently 33 of these (0.7 per thousand) were found to be known to one of the health department agencies, while 84, or 2.1 per thousand, had previously unknown active tuberculosis. In this connection, it should be emphasized that a history obtained from a prisoner is most unreliable since it soon becomes common knowledge among them that anyone with tuberculosis receives special treatment.

There were two sources of follow-up and final diagnosis. Of the original 1,289 tuberculosis suspects, the diagnosis was completed in 426 cases while the prisoner was still in the county jail (table 4). Tuberculosis, both active and inactive, was diagnosed in 270 persons (including the 143 persons charged with violation of isolation order), while in 156 cases tuberculosis was excluded. The remaining 863 suspects were referred to the health department for follow-up primarily because they were released from custody before diagnostic procedures could be completed. Of these, 334 were subsequently found to have tuberculosis. The health department was unable to locate 190 of these "suspects" for such reasons as the person's having left the county or the use of false names and addresses. In another 225 cases the diagnosis was incomplete. In some of these, the diagnosis is still pending, and some of the ex-prisoners have been referred to other jurisdictions for final diagnosis. Follow-up, therefore, was completed in 874 of the 1,289 suspected cases, or in 68 per cent of the group.

In table 5 a comparison is made of the stage of disease in those with previously known and unknown tuberculosis. Whereas 22.6 per cent of the patients with previously unknown tuberculosis had minimal disease, 7.4 per cent of those with previously known disease had minimal lesions. While there were 29.6 per cent of moderately advanced cases among those with previously known disease, 42.8

per cent of the previously unknown cases were moderately advanced. The most striking contrast is seen in the far advanced group which included 60.8 per cent of the previously known cases but only 27.4 per cent of the previously unknown cases. It is quite apparent, therefore, that among the previously known cases, more than one-half were far advanced, while in the newly discovered group two-thirds were minimal or moderately advanced.

TABLE 4

SOURCE OF FINAL DIAGNOSIS

Total tuberculosis suspects	1,289		
Final diagnosis in jail		426	
Tuberculosis			270
Not tuberculosis			156
Referred to health department		863	
Tuberculosis			334
Not tuberculosis			114
Unable to locate			190
Diagnosis not complete or pending			225

TABLE 5

CASES OF PREVIOUSLY KNOWN AND PREVIOUSLY UNKNOWN ACTIVE DISEASE BY STAGE OF DISEASE

Stage	Previously Known		Previously Unknown		Total	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Minimal	13	7.4	19	22.6	32	12.3
Moderately advanced	52	29.6	36	42.8	88	33.9
Far advanced	107	60.8	23	27.4	130	50.0
Not stated	4	2.2	6	7.2	10	3.8
Total	176	100.0	84	100.0	260	100.0

TABLE 6

SEX AND RACE DISTRIBUTION FOR ALL CASES OF TUBERCULOSIS, ACTIVE TUBERCULOSIS, AND PREVIOUSLY UNKNOWN ACTIVE TUBERCULOSIS

Racial Group	All Persons with Tuberculosis						Persons with Active Tuberculosis						Persons with Previously Unknown Active Tuberculosis					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
White																		
Latin American	173	19.3	4	10.1	177	19.0	78	8.7	2	5.1	80	8.6	24	2.7	0	—	24	2.6
Other	323	13.9	34	14.4	357	14.0	130	5.7	14	5.9	144	5.7	48	2.1	4	1.7	52	2.0
Negro	61	11.1	5	7.3	66	10.7	30	5.5	3	4.4	33	5.4	5	0.9	1	1.5	6	1.0
Other	4	19.4	0	—	4	11.4	3	14.6	0	—	3	8.6	2	9.7	0	—	2	5.7
Total	561	14.8	43	12.5	604	14.6	241	6.4	19	5.5	260	6.3	79	2.1	5	1.5	84	2.1

TABLE 7

ALL ACTIVE CASES OF TUBERCULOSIS
Rate per 1,000 Persons Examined

Age	Male					Female					Grand Total
	White		Negro	Other	Total	White		Negro	Other	Total	
	Latin American	Other				Latin American	Other				
years											
10–19	4.0	0.4	—	—	1.5	—	—	—	—	—	1.5
20–29	8.4	1.8	4.4	—	4.2	10.7	5.0	6.1	—	6.2	4.4
30–39	8.1	3.8	6.4	—	5.0	—	6.8	4.6	—	5.7	5.1
40–49	16.8	11.2	9.4	78.9	12.2	—	7.9	—	—	6.6	11.6
50–59	13.3	15.0	8.2	—	14.1	—	5.0	—	—	4.3	13.4
60–69	12.3	9.3	15.9	—	10.1	—	—	—	—	—	9.6
70+	64.5	5.6	—	—	13.8	—	—	—	—	—	13.2
Total	8.7	5.7	5.5	14.6	6.4	5.1	5.9	4.4	—	5.5	6.3

The racial distribution, as shown in table 6, indicates that the prevalence rates were highest in persons of Latin American descent; viz., 190.0 per thousand for all types of tuberculosis, 8.6 per thousand for active tuberculosis, and 2.6 per thousand for previously unknown active tuberculosis. Of considerable interest was the fact that the lowest rates were found in Negroes. Although this may be contrary to the general impression, Russakoff (6) had a similar experience.

Age distribution for all persons with active tuberculosis is given in table 7. It is observed that the prevalence rate is considerably higher in the age group of more than forty. This is in accordance with findings of other mass and hospital surveys.

DISCUSSION

Until recently the emphasis of tuberculosis control programs has been centered on mass surveys, mobile roentgenographic units, and hospital admissions, while a segment of the population with one of the highest rates of infection has been somewhat neglected. Continuous case-finding programs in prisons offer a fertile field for the control of tuberculosis for several reasons. The increased prevalence rate of the disease in prisoners makes such programs much more rewarding than are those which are carried out in the general population. Zahn and associates (7) found, in parallel surveys in Seattle, 7.5 active cases per thousand persons among prisoners, compared with a rate of 0.94 per thousand in the general population. The present study reveals a total prevalence rate of 6.3 cases of active tuberculosis per thousand persons, of which 2.1 cases per thousand were previously unknown. The latter is several times the 1955 new case rates of 0.66 per thousand for Los Angeles County and 0.95 per thousand for the city of Los Angeles.

This rate of 2.1 previously unknown active cases per thousand may also be compared with the rate of 6 cases of previously unknown active tuberculosis per thousand found at the Los Angeles County Hospital (8) which draws on a population similar in social and economic background to that of the county jail.

From this one may assume that the yield of newly discovered cases of active tuberculosis from such a program will be at a level between that of the general population and a large general hospital. At this point it should be noted that the Los Angeles County Jail does not, for the most part, collect its patrons from the "Skid Row" population. Institutions which have a greater proportion of such clientele may be expected to show an even higher rate.

Another advantage of a jail program is that one is dealing with a captive population and, therefore, a total survey can be achieved. Although a prisoner cannot be forced to submit to examination, to date none in the present program has objected. This means that even those who may suspect that they harbor disease and prefer not to find out about it are nevertheless included in the examinations.

It has already been shown by Beatty and Horst (4) that a substantial decrease in the prevalence rate of tuberculosis among prisoners can be expected following the institution of a control program. This will result in decreased exposure of jail personnel.

A tuberculosis hospital is an integral part of the program at the Los Angeles County Jail. This must be so if such programs are to be completely effective. The mere finding of a new case of active tuberculosis is of limited value. Adequate treatment facilities must be provided.

The cost per new active case discovered in the present program was $332, a figure somewhat higher than the $180 per new case discovered by the Los Angeles County Hospital Admission Program (8), but considerably lower than the cost per case, $808, in the Los Angeles County mass survey.

SUMMARY

The results of the first year of operation of the Los Angeles County Jail Chest Screening Program are reported. A total of 14.6 persons per thousand examined were found to have tuberculosis, with a rate of 6.3 active cases per thousand persons examined. Previously unknown active tuberculosis was found in 2.1 persons per thousand, which is more than twice the rate for the city of Los Angeles and more than three times the rate for Los Angeles County. More than one-half of the previously known cases were classified as far advanced while in the newly discovered group two-thirds had minimal or moderately advanced disease. The highest prevalence was in the Latin American group. The next highest rate was found in other whites, and the lowest rate was in Negroes. Tuberculosis, both active and inactive, was most common in those more than forty years of age.

It is emphasized that detection of a new case of tuberculosis is only part of an effective program. Treatment facilities and follow-up programs must be provided.

SUMARIO

Plan para el Despistaje Radiográfico del Tórax en la Cárcel del Condado de Los Angeles

Preséntanse los resultados del primer año de funcionamiento del Plan de Despistaje Torácico en la Cárcel del Condado de Los Angeles. Se descubrió que tenía tuberculosis un

total de 14.6 personas en cada mil examinadas, con un coeficiente de 6.3 casos activos por mil examinadas. Se descubrió tuberculosis previamente desconocida en 2.1 personas por mil, lo cual es más del doble del coeficiente para la ciudad de Los Angeles y más del triple del del Condado de Los Angeles. Más de la mitad de los casos previamente conocidos fueron clasificados como muy avanzados, en tanto que en el grupo recién descubierto dos terceras partes tenían tuberculosis mínima o moderadamente avanzada. La mayor frecuencia correspondió al grupo latino-americano. El coeficiente más alto siguiente fué en otras personas blancas, y el más bajo en sujetos de color negro. La tuberculosis, tanto activa como inactiva, fué más común en personas de más de cuarenta años de edad.

Reconócese que el descubrimiento de un nuevo caso de tuberculosis no representa más que parte de un plan eficaz. Hay que suministrar medios de tratamiento y obras de observación subsiguiente.

RESUME

Programme de radiographie systématique du thorax à la prison du Comté de Los Angeles

Les résultats de la première année de la mise en oeuvre d'un programme de dépistage systématique radiologique à la prison du Comté de Los Angeles sont communiqués. Il a été découvert un nombre total de 14,6 cas de tuberculose pour 1.000, dont 6,3 cas en activité sur 1.000 malades examinés. La tuberculose antérieurement non décelée a été découverte chez 2,1 sujets pour 1.000, soit deux fois plus souvent que la proportion enregistrée dans la ville de Los Angeles et trois fois plus pouvent que la proportion enregistrée dans le Comté. Plus de la moitié des cas antérieurement diagnostiqués était classifiée parmi les cas très avancés tandis que le groupe des cas nouvellement découverts variait entre des cas à minima ou modérément avancés. C'est dans le groupe Americano-Latin que la prédominance de la tuberculose était la plus élevée. Le taux dont l'élévation venait immédiatement après s'observait chez les Blancs et le taux le plus bas chez les Noirs. La fréquence de la tuberculose stabilisée ou en activité était plus élevée chez les sujets ayant plus de quarante ans.

Les auteurs insistent sur le fait que le dépistage de nouveaux cas de tuberculose ne représente qu'une partie d'un programme efficace de lutte antituberculeuse. Un programme comportant des facilités de traitement et d'observation des cas traités doit être assuré.

Acknowledgments

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RETENTION AND DIFFERENTIATION OF CARBOLFUCHSIN-STAINED MYCOBACTERIA IN DIAGNOSTIC FILMS[1]

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INTRODUCTION

Sensitive and reliable methods for recognizing acid-fast bacilli are indispensable in the study of all mycobacterial diseases. The numerous formulas for carbolfuchsin solutions and the continued publication of new staining methods indicate, however, a general uncertainty that the desired goal is being achieved. They suggest also that certain basic aspects of the problem have not yet been elucidated.

A step by step analysis of factors which control the retention and differentiation of mycobacteria has thrown much light on this problem. It has been possible, for example, without tedious enumerations of organisms, to define the role of proteins as filming agents which minimize the loss of mycobacteria from slides, the fixative or disruptive effects of reagents, and the end points which designate proper degrees of differentiation. Enumerations of stained mycobacteria reveal that decolorizing agents differ considerably in their selectivity. It is clear, therefore, that a number of variables other than carbolfuchsin stains may be primary determinants of the reliability and sensitivity of a staining procedure.

Fortunately, the major difficulties in demonstration of the mycobacteria can be avoided by simplification of reagents and procedures. Observations bearing on these questions as they relate to diagnostic films are reported in the present paper. Emphasis will be placed on the reasons for less than optimal results in popular methods and on the distance between the results achieved in practical work and a 100 per cent reference standard.

MATERIALS AND METHODS

Mycobacteria: On a practical basis, comparisons of the numbers of mycobacteria demonstrable in stained preparations have been made in clinical specimens, cellular exudates, and tissue homogenates containing *M. tuberculosis*, *M. lepraemurium*, or *M. leprae*. Washed suspensions of human and murine leprosy bacilli and pure cultures of tubercle bacilli and of the saprophytic species (usually the HMS strain of *M. phlei*) have been enumerated when suspended in body fluids or ingested by phagocytic and cultivated tissue cells. If the microorganisms had not been "declumped," all clusters were counted as a single unit.

Sampling methods: Nonhomogenized sputums, exudates, and similar specimens were drawn out in one or more horizontal streaks across the slide. If necessary, water was added while random distribution was improved by rapid vibratory strokes with a stiff loop until the specimens "flowed" like a smooth varnish. The final stroke was "tailed out" of the

[1] From the Leonard Wood Memorial Laboratory, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts.

TABLE 1

STAINING AND DIFFERENTIATING SOLUTIONS

Designation of Reagents	Stock Solutions	Volumes Combined	Final Concentrations
		ml.	*per cent*
1. Carbolfuchsin	a) 3% basic fuchsin (F) in 95% alcohol (A)	10	F = 0.3
	b) 5% phenol (P) in distilled water	90	A = 9.5 P = 4.5
2. Sulf,MB	c) 1% aqueous methylene blue (MB)	20	MB = 0.2
	d) concentration of sulfuric acid (Sulf) 5% vol. per vol.	80	Sulf = 4.0
* MB[6]	$M/30KH_2PO_4$ } pH 6.0 $M/30Na_2HPO_4$ }	90 10	
	c) 1% aqueous methylene blue (MB)	5	MB = 0.05
* MB[6.5]	$M/30KH_2PO_4$ } pH 6.5 $M/30Na_2HPO_4$ }	70 30	
	c) 1% aqueous MB	5	MB = 0.05

1. Always add the phenol solution to the alcoholic basic fuchsin. The working solution should appear clear by transmitted light and opaque by reflected light. Store in a tightly sealed bottle in an incubator; add a drop or two of alcohol as required to disperse any surface scum.

2. Subnumerals indicate percentage concentrations of concentrated sulfuric acid vol. per vol.

* Not recommended for diagnostic purposes. Use as counterstains for thirty seconds only when it is necessary to impart coloration to the cytoplasm of cells, e.g., in phagocytic preparations, cell cultures, or tissue sections. Exponents indicate pH.

strip to be examined. In the case of preparations from phagocytic studies, a small loop of cell suspension was drawn along the slide with a single stroke of a spreader which was 5 mm. wide. As indicated below, vertical divisions facilitated the study of variables.

Enumerations of stained mycobacteria in more homogeneous samples were made across the diameters of circular films prepared from constantly stirred suspensions as follows: 0.01 ml. samples on 16 mm. circles (1); 0.0025 ml. (2 mm. standard loop) on 8 mm. circles; and 0.000, 22 ml. (0.7 mm. standard loop) as "microspots" which were allowed to spread to their natural limits on clean slides. If "microspots" of comparable diameter are used, this method is preferred. A large number of such spots can be arranged in horizontal rows on glass slides laid on graph paper. Subsequent rows can carry bacilli of different species or specimens from different sources.

After slides bearing films in horizontal rows had received the desired preliminary treatments (for example, fixation and carbolfuchsin staining), they were rinsed in water and dried. They were then warmed slightly so that vertical strokes of a wax pencil partitioned the slide for different methods of decolorization or differentiation. Rinsings were drained from the slides tilted sidewise onto plaques of paper towels. Such procedures permit also the application of variables prior to a uniform final treatment. They assure that all treatments but the intended variables are comparable.

Reagents: Two working solutions suffice for a broad variety of diagnostic purposes. The four stock solutions and the methods of combination are indicated in table 1. This table shows also the counterstains which may be useful in other types of investigation. The

composition of other reagents is indicated in the text or as footnotes to tables. All reagents are used directly from stock or dropping bottles without disturbing the sediments.

Staining procedure for diagnostic spreads on glass slides: The slide is placed on a hot plate over boiling water for thirty seconds;[2] the carbolfuchsin is applied for thirty or sixty seconds; and the slide is rinsed *gently* in water. The preparation is differentiated in $Sulf_4MB$ as required (usually thirty to sixty seconds), tilting the slide at least once, and is then rinsed *gently* in water.

The $Sulf_4MB$ differentiator permeates and extracts dense portions of irregular films, without decolorization of mycobacteria. The lowest effective strength of concentrated sulfuric acid is 0.4 per cent vol. per vol.; 1 per cent proved satisfactory for thin, uniform films. A single supply of $Sulf_4MB$ is used for months in sealed Coplin jars or in vat methods.[3] Whether in Coplin jars or applied to slides, all differentiators should be agitated at least once to promote uniform exchange of colors. The methods used to elucidate specific questions will be indicated under the appropriate topics.

OBSERVATIONS

Many observations prevent assigning a special role to the various formulations of carbolfuchsin solutions. Because of their fixative action, carbolfuchsin solutions may be applied directly to spreads of cultivated mycobacteria or to those washed from tissues. After rinsing with water, or a single rinse in 95 per cent alcohol, no dense background remains to interfere with microscopic examination. All solutions, except when deteriorated, stained mycobacteria adequately. It is unnecessary to employ supersaturated, unstable solutions and unwise to apply any solution so long that it complicates the distinction of mycobacteria from the background in which they are embedded.

Factors Which Reduce the Loss of Mycobacteria from Slides

Protein films: The tendency for mycobacteria to be lost from the surface of the diagnostic type of films during staining procedures has been demonstrated (2). The proper use of protein filming agents was reinvestigated by employing comparable amounts of dilute protein solutions (usually serum 5 per cent) in three ways: (*a*) as dried circular films on which mycobacterial suspensions were spread, (*b*) mixed with the suspensions prior to making the spreads, and (*c*) as surface coats applied to previously dried mycobacterial spreads. The first arrangement will be recognized as the one commonly employed with filming agents

[2] The heat required barely suffices to liberate vapors; prolonged heating creates more difficulties than benefits.

With appropriate differentiation, staining at room temperature for five minutes has been as satisfactory as the use of heat. Since the carbolfuchsin solution serves as a fixative, no preliminary fixation is necessary.

[3] Evidence that bacilli are lost from the surface of films must not be taken to support theoretical objections to vat staining. Many hours have been spent searching blank protein films on slides which were left in Coplin jars while processing slides carrying large numbers of washed *M. leprae* which were not embedded in protective films. Mycobacteria could not be found on the blank films. The prompt agglutination of mycobacterial suspensions by carbolfuchsin and by the differentiators suggests that neutralization and/or reversal of charge prevents adhesion of the bacilli to blank preparations. Information including any actual data on the successful demonstration of bacilli on blank slides placed in staining vats would be appreciated by the writer.

such as Mayer's albumin. Published data (2) and subsequent experience indicate that for each 60 acid-fast bacilli which can be demonstrated after exposure on the surface of protein films, 80 are found when embedded within the protein films, and 100 when protein is used as a protective coating.[4]

These tedious counts can be replaced by transillumination of uniform films prepared from bacterial suspensions or from body fluids containing india ink, 1 per cent vol. per vol., or nigrosin, 0.5 per cent. After exposure to reagents singly or in series, the loss of opacity identifies factors which disrupt the bonding of proteins and films to slides. For example, the loss of pigment following the use of protective reagents indicates whether the natural protein content of a natural body fluid provides adequate films. It readily becomes apparent that mycobacteria from pure cultures, those washed from tissues, or those in specimens digested with trypsin or pancreatin require an admixture of fresh protein to prevent the loss of a substantial proportion of the microorganisms.

The Protective or Disruptive Effects of Individual Reagents

No method of protein filming or of fixation prevents loss of mycobacteria if the reagents used in staining or differentiation can disrupt protein films and tissue cells.

Information concerning the fixative or destructive effects of individual reagents was obtained by exposing thin, unfixed, unstained films of 50 per cent blood for three minutes to: carbolfuchsin; 95 per cent alcohol at pH values of 5, 6, and 6.5; sulfuric acid, 5 per cent vol. per vol.; 2.5 per cent potassium alum sulfate in sulfuric acid, 2.5 per cent vol. per vol.; M/30 and M/100 citric acid; water; and for fifteen seconds to 3 per cent HCl-alcohol. After rinsing in water and counterstaining with MB[6.5] (table 1) for sixty seconds, results were assessed by recording the preservation or lysis of erythrocytes and leukocytes and also the effects upon the thin margins of plasma films.

Protection of cells and films occurred in carbolfuchsin, in alcohol buffered at mild acidities, and in the acidified sulfates. Etching and wrinkling of the plasma films and distortion or destruction of cells occurred in water and citric acid and particularly in acid-alcohol. After hot staining with carbolfuchsin for thirty seconds, cold staining for five minutes, or one to two minutes' fixation in 95 per cent alcohol, water or citric acid was tolerated for long periods. Heat-, alcohol-, formalin-, and carbolfuchsin-fixation, alone or combined, failed to protect against the disruptive effects of brief exposure to acid-alcohol. Thus, three classes of reagents were distinguished: (*a*) those with fixative effects: carbolfuchsin, buffered alcohols, and acidified sulfates; (*b*) nonprotective reagents: water and citric acid; and (*c*) the destructive reagent, acid-alcohol.

[4] As a convenient, stable surface-coater, a thin film of 0.5 per cent gelatin in 0.5 per cent phenol has been adopted for standard counts in experimental work, even when mycobacteria are already suspended in dilute serum or body fluids. A thin surface coat is applied with a spreader, dried, then hardened by exposure for three minutes to formalin fumes. Formalin increases the basophilism of the bacilli and the proteins so markedly that differentiators must be applied for five minutes. Although this method serves as a reference standard for the practical problems under consideration, it is too cumbersome for routine diagnostic procedures.

All available enumerations of mycobacteria, irrespective of the type of specimens or samples, support these conclusions.

The Selective Action of Decolorizers and Differentiators

If protein films and protective reagents minimize the loss of mycobacteria from slides, reduction of the number of reagents and of rinsings should be advantageous. The properties of decolorizers are not modified by low concentrations of methylene blue. This modification reduces reagents and rinsings by providing *differentiators* which counterstain while decolorizing. The presence or absence of methylene blue, however, may be ignored while reviewing the selectivity of decolorizing agents.

Alcoholic solutions: After many comparisons with alcohol containing decreasing concentrations of acid and, finally, buffers of diminishing H-ion concentration, alcohol finally was converted to a differentiator which possessed interesting merits in competition with other differentiators. When 5 ml. of M/30 PO_4 at pH 5 were added to each 100 ml. of 95 per cent alcohol containing 0.05 per cent methylene blue, unusually dense films and stains were differentiated within sixty seconds. Addition of the same buffer at pH 6 was superior for routine diagnostic purposes, while pH 6.5 imparted a desirable tint to the cytoplasm of leukocytes and of cells cultivated *in vitro*.

These alcoholic differentiators are superior to the two-step procedure involving HCl-alcohol followed by dilute methylene blue. Nevertheless, they cannot be recommended for diagnostic work for two reasons. The carbolfuchsin complex in saprophytic species and in *M. leprae* in paraffin sections from old lesions and treated patients is replaced about as readily as from tissue cells and proteins; a bluish tint is usually imparted to the organisms. Even with pathogenic species in a presumably average physiologic state, no counts or comparisons have equaled the results with sulfuric differentiation (tables 2, 3, and 4).

Citric acid: M/100 citric acid containing 0.05 per cent methylene blue differentiated most materials within one minute, while M/30 sufficed for the most

TABLE 2

COMPARISON OF ACID-ALCOHOL *versus* A SULFURIC DIFFERENTIATOR

Tubercle Bacilli in Sputum Spreads

Decolorization or Differentiation	Observer		Total Bacilli	Relative Numbers
	A	B		
3% HCl in alcohol, then counterstain with methylene blue	154	161	315	76
Methylene blue 0.2% in sulfuric 5% by volume	212	204	416	100

Trypsin-digested sputums from 10 patients were spread on paired slides, all of which were stained simultaneously; individual slides of each pair were decolorized by the respective methods; enumerations were conducted without knowledge of treatments: 50 fields per slide except that, if 50 bacilli were seen in any slide, counting was terminated and the same number of fields was observed in the companion slide.

TABLE 3

COMPARISON OF ACID-ALCOHOL *versus* ALCOHOLIC AND SULFURIC DIFFERENTIATION
Declumped Tubercle Bacilli from a Sputum Concentrate

Reagent	A	B	Total Bacilli	Relative Numbers
a) 3% HCl alcohol ten seconds b) MB[0.05] thirty seconds	258	234	492	76
0.05% methylene blue in alcohol pH 6.5, sixty seconds	269	284	553	86
Sulf.MB, sixty seconds	314	333	647	100

Notes: Sputum homogenized in N/2 NaOH and neutralized with N/2 HCl; bacilli declumped, collected with chloroform 10 per cent; bacilli suspended in 0.1 per cent serum albumin; filmed in microspots. Fixation: hot plate one minute. Staining: hot plate thirty seconds. Each determination, each observer: bacilli per 100 fields.

* *See* table 1.

TABLE 4

COMPARISON OF ALCOHOLIC AND SULFURIC DIFFERENTIATORS
M. Lepraemurium in Fibrocytes Cultivated *in vitro*

Reagent	Per Cent Cells with Bacilli	Bacilli per 100 Cells	Relative Numbers of Bacilli
0.05% methylene blue in alcohol pH 4, three minutes	89	954	79
0.05% methylene blue in alcohol pH 6, six minutes	92	995	83
Sulf.MB, three minutes	100	1,200	100

Notes: Infected cells cultivated on a series of micro coverslips, all in the same liquid medium. Fixation: methyl alcohol five minutes. Staining: hot plate thirty seconds.

difficult materials. Citric acid resembled alcohols in its failure to effect discriminatory discharge of carbolfuchsin.

Mineral acids: The concentrated mineral acids at 5 per cent vol. per vol. provide approximately 0.8N HNO_3, 0.6N HCl, and 1.8N H_2SO_4. Following application for one to three minutes, the order of excellence was: $H_2SO_4 > HCl > HNO_3$. Nitric and hydrochloric acids are destructive to cells and filming proteins as well as deficient in differential action.

Acid sulfates: The protective action of sulfates on cells and films during exposure to low pH apparently is due to the property which has made sulfates the ion of choice for washing out mineral acids after decalcification of bone for histologic purposes. In addition, acid sulfates exhibited the most selective action yet demonstrated for discharging carbolfuchsin from tissue components while preserving the stain in mycobacteria.

Potassium alum sulfate 5 per cent wt. per vol. did not decolorize completely within twenty-four hours. Ferric ammonium sulfate 5 per cent wt. per vol., the mordant for Heidenhain's iron-hematoxylin, produced within twenty-four hours

the most satisfactory decolorization of any reagent investigated. Either alum solution mixed with an equal volume of sulfuric acid 5 per cent vol. per vol. discharged the carbolfuchsin from tissue components at rapid and dependable rates. Sulfuric acid is recommended for diagnostic purposes because it is convenient, both as a diluent for methylene blue and as an effective source of hydrogen and sulfate ions.

Alcoholic versus Sulfuric Differentiators

The enumerations of *M. tuberculosis* and of *M. leprae* are from the diagnostic type of films; those of *M. lepraemurium* and *M. phlei* concern mycobacteria within tissue cells.

Tubercle bacilli: A practical comparison between decolorization in acid-alcohol and differentiation in dilute sulfuric acid was made in collaboration with Mrs. Joan B. Daniels of the Massachusetts State Health Laboratory (table 2).

"Declumped" tubercle bacilli from sputum filmed in standard "microspots" provided another example of acid-alcohol revealing 76 per cent of the numbers seen after differentiation in $Sulf_4MB$ (table 3). The inferiority of acid-alcohol in comparison with buffered alcoholic differentiators is also illustrated in this table.

M. leprae: Ratios of 70:100 tend to express the relative numbers of *M. leprae* from active lesions which were demonstrable after decolorizing in acid-alcohol and after differentiation in sulfuric 5 per cent by volume containing methylene blue 0.2 per cent (2). In a subsequent communication it will be shown that *M. leprae* in paraffin-embedded sections of treated lesions is so susceptible to acid-alcohol and to alcoholic differentiators that the differences between alcoholic and sulfuric differentiation must be expressed in orders of magnitude.

M. lepraemurium: The most favorable comparisons between alcoholic and sulfuric differentiators were obtained with this pathogenic species under conditions which permitted no mechanical losses of bacilli (table 4). It should be

TABLE 5

COMPARISON OF ACID-ALCOHOL *versus* ALCOHOLIC AND SULFURIC DIFFERENTIATION
M. Phlei in Polymorphonuclear Leukocytes

Reagent	Per Cent Cells with		Relative Numbers: Red Bacilli
	Blue Bacilli	Red Bacilli	
a) 3% HCl alcohol ten seconds...			
b) $MB^{0.5}$ thirty seconds.....	18	7	18
0.05% methylene blue in alcohol pH 6.5, thirty seconds.....	9	22	56
$Sulf_1MB^*$, thirty seconds.....	2	39	100
$Sulf_{0.4}MB^*$, sixty seconds.....	0	38	98

Phagocytosis, fifteen minutes. Fixation, hot plate one minute. Stain: carbolfuchsin on hot plate, one minute. All cells containing one red bacillus were scored in the red column, irrespective of the presence of blue ones.

* Sulfuric 1% and 0.4% vol. per vol., respectively.

noted that alcoholic differentiators become less reliable when the buffer is as low as pH 4.

M. phlei: It must not be concluded that these favorable results with alcoholic differentiators may be depended upon whenever mycobacteria are encased within cells or that acid-alcohol under these circumstances becomes a reliable reagent. An example of the failure of alcoholic decolorization or differentiation to demonstrate any reasonable proportion of total mycobacteria is presented in table 5. Evaluation of the results with *M. phlei* in leukocytes must be based on red bacilli only since, in problems of diagnosis or identification, the blue forms of mycobacteria are regarded as contaminants.

Proportions of Mycobacteria Demonstrated

Confidence in any method depends upon the actual relationship between practical determinations and a theoretically acceptable standard. Such standards are necessary in order to know when methods are deceptively inefficient and when they attain sufficient sensitivity so that further effort toward improvement is warranted only on the basis of new principle.

A reference standard of total mycobacteria which should be demonstrable by staining has been developed to a point where the percentage efficiency of staining methods can be stated. Cultivated or washed mycobacteria are suspended in serum 1 per cent vol. per vol. (or in 0.05 per cent serum albumin) containing 0.5 per cent nigrosin[5] as outlining medium. With the 0.7 mm. loop, "microspots" are prepared on slides in front of a fan. The organisms are then enumerated without having exposed the preparations to any staining or rinsing solution. Similar "microspots," protected or modified as desired, are stained with carbol-fuchsin and differentiated prior to enumerating the bacilli. This reference standard indicates that some 90 to 95 per cent of mycobacteria may be retained and stained beneath a formalinized gelatin coat as described in footnote 4. When the organisms are simply embedded in protein films, as in diagnostic work, some 70 per cent are demonstrated after sulfuric differentiation, e.g., by $Sulf_4MB$. Since relative values of 100 in the foregoing tables represent only 70 per cent of the original concentration of bacilli in the films, the values obtained with pathogenic species after acid-alcohol decolorization were in the range of 50 per cent under the best circumstances shown.

DISCUSSION

Invention of primary mycobacterial stains has flourished for a long time without equal interest in the fact that such stains are only the first step toward a result which is dependent on many factors.

In this effort to analyze the more important determinants of sensitivity and reliability, the usual recitation of specimens positive or of bacilli demonstrated

[5] Stock solution: Hot aqueous extract of nigrosin powder at 2.0 per cent; centrifuged 10' @ 1850 × G; supernate decanted through a paper filter onto a Corning UF sintered glass membrane; both filters rinsed with sufficient water to make a final concentration of 1 per cent in the filtrate; preserved with 2 per cent formalin.

has been avoided. Such data afford comparisons between variables imposed on over-all procedures. They do not, however, afford means of recognizing or analyzing liabilities in reagents or procedures in routine use in busy laboratories. An attempt has been made, therefore, to describe criteria which permit evaluation of filming agents, fixing methods, reagents, and end points of differentiation without enumeration of mycobacteria. Enumerations have been presented only to illustrate the differing selectivities of decolorizing and differentiating agents under optimal conditions.

The evidence indicates that in the routine use of alcoholic decolorizers and differentiators there exists always the possibility of failure to reveal anticipated proportions of the mycobacteria initially present. In the absence of data concerning circumstances in which the resistance of tubercle bacilli to alcoholic decolorizers may be impaired, observations with *M. phlei* and with *M. leprae* from treated patients have been mentioned. In Badger's comparisons (3) of 3 per cent HCl-alcohol versus Gabbett's differentiator (4)[6] in films containing *M. leprae* from scraped skin incisions, the relative numbers of bacilli demonstrated were 40:100. Should these data represent the average results in diagnostic laboratories more faithfully than the data here shown, efficiencies of approximately 30 per cent with acid-alcohol would be the highest estimate justified. Just where this value may fall when filming is less reliable than in spreads from scraped skin incisions and when technicians are unaware of pitfalls becomes, therefore, a matter of interest and concern.

The superiority of the staining method suggested under Materials and Methods probably lies in the fact that films are exposed to a fixative (carbolfuchsin), to water, to a fixative (sulfuric methylene blue), and to water.

Information concerning differentiating reagents has little meaning unless supplemented with knowledge concerning optimal periods of application. End points can be determined without enumerations of stained mycobacteria, provided two facts are accepted: (*a*) that distinction of mycobacteria from backgrounds does not depend on qualitative differences but resembles a titration and (*b*) that differentiation must be controlled by timing. Since protein films and tissue components are preserved by proper reagents, and are of uniform quality, they serve as criteria of end points. Suggestions follow:

> In first trials, withdraw slides from Coplin jars or vats, or tilt those being treated horizontally, in order to determine when a bluish color appears to replace the redness throughout thin blood films, at the margins of microdroplets, in the reasonably dense portions of films of sputum or exudates, or in tissue sections.
>
> After rinsing in water, examine the moist preparations under the low-power objective. The red color should not have been discharged completely from unusually dense portions of sputum, exudates, et cetera. Since these areas cannot be searched satisfactorily, differentiation should be terminated while maximal carbolfuchsin is retained by bacilli in the portions of film which can be examined profitably.
>
> Cornified epithelial cells and spermatozoa should appear slightly red; epithelial cells of sputum and also red cells should exhibit a rosy orange color; films of protein

[6] Methylene blue 2 per cent in concentrated sulfuric acid 25 per cent vol. per vol.

or body fluids should appear colorless. The deliberate addition of a few erythrocytes during standardization for certain specimens, or for checking, may be desirable.

If, with the material at hand, any differentiator passes these end points in less than thirty seconds, the decolorizing component should be diluted to one-fourth of its existing concentration. Once the differentiation time has been established, it should be controlled by timing.

In the use of differentiators two advantages are obvious: the superior retention of bacilli and the saving of time and work. There are, in addition, two more subtle advantages. The cationic methylene blue participates in the ionic exchanges which release carbolfuchsin from cells and background materials. It establishes, therefore, a variety of color tints which assist in the identification of cells and other particulates. Furthermore, the dense portions of films become permeated with the decolorizing agent as well as with counterstain. The presence of a decolorizing agent during the early seconds of rinsing prevents the methylene blue from overstaining those portions of film which often can be examined with great profit. This feature is so useful that the pale counterstains should not be increased for straightforward diagnostic work.

SUMMARY

Optimal demonstration of mycobacteria in diagnostic films does not depend on the primary stain as much as upon other factors:

(*1*) Imbedding or covering the mycobacteria with protective protein films.

(*2*) Reagents which exert fixative rather than disruptive effects on cells and protein films. These properties are possessed by carbolfuchsin solutions, by alcohol buffered at mild acidities, and by acidified sulfates. Acid-alcohol is a destructive reagent.

(*3*) Judicious rather than excessive staining with carbolfuchsin.

(*4*) Decolorizing agents which conserve the carbolfuchsin complex in mycobacteria while displacing it from cells and background components. Acid-alcohol, alcohols buffered at mild acidities, and citric, hydrochloric, and nitric acids are less discriminatory than acidified sulfates.

(*5*) Simplification of procedures by using sulfuric differentiators containing dilute methylene blue.

(*6*) Applying the differentiators at concentrations which permit timing their action to determinable end points.

Means have been described for evaluating the proper use of protective protein films, the fixative or disruptive effects of reagents, and proper degrees of differentiation without enumeration of stained mycobacteria. Enumerations have been cited to illustrate the differing selective properties of decolorizing agents and the probable average efficiency of practical methods.

SUMARIO

La Retención y Diferenciación de las Micobacterias Teñidas con Carbolfuchsina en las Radiografías de Diagnóstico

El descubrimiento óptimo de las micobacterias en las radiografías de diagnóstico no depende de la coloración primaria tanto como de otros factores:

(*1*) Incrustación o revestimiento de las micobacterias con películas protectoras de proteína.

(*2*) Reactivos que ejerzan efecto fijador más bien que desorganizador sobre las células y las películas de proteína. Poseen estas propiedades las soluciones de carbolfuchsina, el alcohol amortiguado a una acidez leve y los sulfatos acidificados. El ácido-alcohol es un reactivo destructor.

(*3*) Coloración juiciosa, más bien que excesiva, con carbolfuchsina.

(*4*) Descolorantes que conserven el complejo carbolfuchsínico en las micobacterias a la par que lo desplazan de las células y los componentes del fondo. El ácido alcohol, los alcoholes amortiguados a una acidez leve y los ácidos cítrico, clorhídrico y nítrico son menos diferenciadores que los sulfatos acidificados.

(*5*) Simplificación de los procedimientos mediante el uso de diferenciadores sulfúricos que contengan azul de metileno diluído.

(*6*) Aplicación de los diferenciadores a concentraciones que permitan acomodar su acción a puntos terminales determinables.

Quedan aquí descritos medios para justipreciar el empleo apropiado de películas protectoras de proteína, los efectos fijadores o desorganizadores de los reactivos y los grados adecuados de diferenciación sin enumeración de las micobacterias teñidas. Se citan enumeraciones que ilustran las diversas propiedades selectivas de los descolorantes y la probable eficacia media de técnicas prácticas.

RESUME

Rétention et Différenciation des mycobactéries colorées à la carbofuchsine dans les films diagnostiques

La démonstracion optima des mycobactéries par les clichés diagnostiques ne dépend pas autant de la coloration primaire que d'autres facteurs:

1) Enrobement ou recouvrement des mycobactéries par des films protéiques protecteurs.

2) Réactifs exerçant une action de fixation plutôt que des effets de dissociation sur les cellules et les films protéiques. Ces propriétés sont possédées par les solutions de carbofuchsine, par l'alcool tamponné à pH acide faible et par les sulfates acides. L'alcool-acide est un réactif destructeur.

3) Coloration modérée plutôt qu'excessive par la carbofuchsine.

4) Agents de décoloration que conservent la carbofuchsine dans les mycobactéries tout en la déplaçant des cellules et des composants des autres substances. L'alcool-acide, les alcools tamponnés à pH acide faible et les acides citriques, chlorhydriques et nitriques permettent moins la discrimination que les sulfates acidifiés.

5) Simplification des procédés par l'utilisation de réactifs sulfuriques de différenciation renfermant du bleu de méthylène dilué.

6) Application des réactifs de différenciation aux concentrations permettant de déterminer le temps de leur action jusqu'à la fin de la réaction (end-points).

L'auteur décrit les moyens utilisés pour évaluer l'usage approprié des films protéiques protecteurs, les effets de fixation ou de dissociation produits par les réactifs et les degrés adéquats de différenciation sans numération des mycobactéries colorées. Les numérations illustrent les différentes propriétés sélectives des agents de décoloration et l'efficacité moyenne probable des méthodes usuelles.

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RETENTION AND DIFFERENTIATION OF MYCOBACTERIA IN TISSUE SECTIONS[1]

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INTRODUCTION

Reliable methods for demonstrating acid-fast bacilli are indispensable in the study of mycobacterial disease. Mycobacteria in tissue sections cannot be demonstrated as reliably as in contact impressions from the cut surfaces of organs. Reasons for the less satisfactory results in sections have been examined.

In the present report it is proposed to set forth evidence of the mechanical loss of mycobacteria from tissue sections during the usual deparaffinization procedures; to demonstrate that the acidified sulfate differentiators, optimal for diagnostic films (1), are equally valuable for decolorizing and differentiating mycobacteria in paraffin-sectioned tissues; and to suggest that, for many purposes, cytologic methods are more useful than histologic methods in the microscopic study of mycobacterial lesions.

MATERIAL AND METHODS

The writer is indebted to Dr. Herman Gray, formerly of the Marine Hospital, Carville, Louisiana, for a generous supply of paraffin-mounted sections of formalin-fixed skin from leprosy patients who had been treated with sulfones until the numbers of demonstrable mycobacteria had apparently become negligible; also to Dr. G. Dammin, Pathologist of the Peter Bent Brigham Hospital, Boston, Massachusetts, for Zenker-fixed sections of a human lung exhibiting miliary tuberculosis.

Deparaffinization: For comparative purposes, sections have been deparaffinized in several ways:

1. *Rapidly:* by immersing them twice in xylene or benzene.
2. *Slowly:* by immersing them in xylene and mineral oil (2) and in turpentine and mineral oil (3).
3. *Slowly:* two or more loops of xylene were applied in succession to the marginal paraffin of each section and then spread gently over its surface. Three minutes were permitted for permeation of the section with solvent and dissolved paraffin.
4. *While staining by Wardekar's visionary method*[2]*:* slides were placed on the hot plate (over boiling water) and flooded with carbolfuchsin, which was replaced

[1] From the Leonard Wood Memorial Laboratory, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts.

[2] Upon being presented with evidence that the lepromatous sections contained more bacilli than could then be demonstrated, Dr. R. V. Wardekar said: "We must be visionary!" Within a few minutes he suggested the removal of paraffin by immersing slides in boiling water (skimming the paraffin) and by the method described. Although these procedures do not effect a uniform removal of paraffin from sections, they were the first to show that myriads of brilliantly stained bacilli could be revealed after proper differentiation. These "visionary" methods permitted elucidation of several important points.

repeatedly while the molten paraffin was skimmed from the surface of the hot carbolfuchsin. They were then transferred directly to water and differentiated.

In the first three methods of deparaffinization, the solvents or solvent-oil mixtures were displaced in either of two ways: (*1*) The end of the slide was laid on a pencil while a slow flow of xylene was allowed to pass slowly over and through the sections in two separate applications, each blotted off the lower ends of the slides promptly and completely. Carbolfuchsin was applied directly; final traces of solvent were removed as vapor during staining. (*2*) Slides were rinsed twice with xylene, which was followed by alcohol, then by carbolfuchsin.

Staining: Carbolfuchsin was applied directly at room temperature; the slides were laid on a hot plate over boiling water for one minute (1), then rinsed in water.

Differentiation: The water-rinsed slides were warmed and dried sufficiently so that vertical strokes of a wax pencil could be applied, thus making it possible to decolorize or differentiate each of the three or four sections on a slide by separate treatments (1). Decolorization and differentiation were controlled by criteria previously described (1). After a final gentle rinse in water, the sections were allowed to become permeated with Schillaber's immersion oil as they approached dryness. They were examined directly under oil.

Methods for examining specific questions can most readily be indicated in relation to specific observations.

OBSERVATIONS

In tissue sections from sulfone-treated leprosy patients, many bacilli tend to be collected in lakes or pools of varying size. Each is surrounded by a multicellular capsule which has been sectioned like the rind of a fruit. The precarious anchorage of the numerous bacilli permits investigating factors which release mycobacteria from sectioned structures.

The Loss of Bacilli from Sections of Lepromatous Tissue

The staining procedure and the selective differentiators employed made it unlikely that the small numbers of mycobacteria observed after deparaffinization in xylene or benzene, especially when alcohol was used to replace these solvents, could be explained by failure to stain or differentiate properly. The residual bacilli were consistently red. Furthermore, the increased numbers of open holes (vacant globus sites) in the sections suggested that the majority of the bacilli had been lost.

This premise was substantiated in two ways:

(*1*) By demonstrating abundant bacilli in the paraffin rinsings obtained with these solvents. The rinsings were dried down on clean slides, stained by Wardekar's method, and differentiated in 0.2 per cent methylene blue in sulfuric acid 4 per cent by volume. (*2*) By demonstrating that the myriads of bacilli retained in properly deparaffinized, stained, and differentiated sections were readily released by certain reagents. A loop of xylol, then one of alcohol, or a loop of water followed by one of alcohol, was applied to the margin of such sections. Stained mycobacteria were found where the interacting solvents dried down after sweeping across the sections.

Losses from Sections Containing Modest Numbers of Mycobacteria

In tuberculosis and in nonlepromatous leprosy the numbers of mycobacteria are much lower than in lepromatous lesions. The ratio of intact cells and cell

TABLE 1

RELATIVE NUMBERS OF TUBERCLE BACILLI DEMONSTRABLE AFTER RAPID AND AFTER CAUTIOUS DEPARAFFINIZATION OF ADJACENT SERIAL SECTIONS

	Microscopic Counts in 12 Analogous Structures		
	A. Xylene, 2 Minutes; Xylene Rinse Twice; Alcohol Rinse Twice*	B. Xylene 20%, Mineral Oil 80%—15 Minutes; Xylene Rinse Twice*	Difference in Numbers
	12	13	+1
	8	13	5
	11	17	6
	129	162	33
	141	138	(−3)
	20	21	1
	7	32	25
	207	301	94
	13	27	14
	1	3	2
	2	12	10
	7	12	5
Total:	558	751	(196 − 3 = 193)
Per cent:	100	134	

* Both: covered with carbolfuchsin at room temperature, heated one minute; water; differentiated 1.5 minutes in 0.2% methylene blue in sulfuric acid 4% by volume; water; mount in oil. Counts restricted to alveoli which could be mapped because of similar form and size in each section.

cytoplasm is higher. It is a fair question whether the observations cited have any significance for such sections.

For quantitative inquiry it was necessary to find infected tissues exhibiting a reasonably uniform distribution of mycobacteria in delineated structures which could be mapped and examined in adjacent sections. The exudate lying in the alveoli of a human lung involved with miliary tuberculosis finally was employed. The numbers of tubercle bacilli observed following (*a*) rapid deparaffinization and displacement of the solvent with alcohol and (*b*) gentle deparaffinization (no alcohol) are illustrated by the series of counts shown in table 1.

The loss of bacilli during deparaffinization was less striking than in the lepromatous tissues. However, it was only once in twelve paired counts that the results did not favor cautious removal of paraffin. By employing slow deparaffinization, 34 per cent more bacilli were demonstrated.

The Causes and Control of Bacillary Losses

There are two causes for release of mycobacteria from sectioned tissues: violent disturbances during rapid deparaffinization and interactions between alcohol and solvents or alcohol and water.

The low-power objective permits observation of the violent surging and "chewing" action which occurs as the advancing margins of liquid xylene or

benzene invade paraffin films. These disturbances seem to liberate innumerable tiny particles from the sections, creating the impression that one is watching a distant swarm of very tiny bees. Even more disruptive wave fronts sweep across the sections when alcohol is used to displace these solvents.

The disruptive action of acid-alcohol on the tissue cells and protein films which attach mycobacteria to glass slides has been described (1). A foregoing section shows that interactions between 95 per cent alcohol and water-wet films are a liability in tissue sections. It must be expected, therefore, that alcoholic differentiators will discharge phagocyted particles from sections more readily than will aqueous reagents.

Violent action of solvents on paraffin can be avoided in either of two ways: by using oil-solvent mixtures (2, 3) or by applying limited volumes of the less volatile solvents so that the paraffin in the sections themselves remains viscid. Slow permeation and extraction of paraffin does not seem to liberate bacilli. As indicated, the merit of these procedures is lost if alcohol is used to displace the solvent system and is minimized if alcoholic reagents are applied to water-wet sections.

The Selectivity of Decolorizers and Differentiators

The discriminating action of decolorizing and differentiating reagents has been evaluated by enumerating mycobacteria in diagnostic films and within intact tissue cells (1). These reagents included: 3 per cent HCl-alcohol; 95 per cent alcohol containing 0.05 per cent methylene blue and phosphate buffers of pH 5, 6, and 6.5; M/100 and M/30 citric acid; the mineral acids, HNO_3, HCl, and H_2SO_4; potassium alum sulfate and iron ammonium sulfate; and each of the latter combined with sulfuric acid. Counts after alcoholic differentiators at pH 5 and 6 were consistently superior to those after acid-alcohol and consistently lower than those in sulfuric acid and the acid sulfates. As the latter reagents excelled in reliability as well as sensitivity, 0.2 per cent methylene blue in concentrated sulfuric acid 4 per cent by volume was suggested as a convenient differentiator for diagnostic purposes.

A comparative enumeration of bacilli in sections of lepromatous tissue is impossible. The results achieved by alcoholic and sulfuric differentiators in sections from sulfone-treated patients, however, differed so greatly that arrangement in order of excellence is justified.

When paraffin was removed gently, i.e., by solvent-oil mixtures or minimal xylene, and sections were stained as described, the average results were: after 3 per cent HCl-alcohol and pale counterstaining, vacant globi and few, if any red bacilli; after 0.05 per cent methylene blue in alcohol buffered at pH 6, many more bacilli, some slightly red, and some blue; after 0.2 per cent methylene blue in sulfuric acid 4 per cent by volume, myriads of bright red bacilli. If paraffin was removed by rapid or improper methods, there remained much smaller numbers of bacilli to be demonstrated after differentiation by each method. Disappointments with alcoholic reagents were assignable to two causes: loss of bacilli and less discriminatory discharge of the carbolfuchsin.

Sections which seemed to contain no mycobacteria after acid-alcoholic decolorization and after alcoholic differentiation permitted rechecking the superiority of sulfuric differentiation in two ways: (*1*) The immersion oil was removed from some by rinsing gently with xylene, carbolfuchsin was applied directly, and the sections were restained. (*2*) The oil was removed from others by Wardekar's method. Following differentiation of each type of such preparations in 0.2 per cent methylene blue in sulfuric acid 4 per cent by volume, moderate to high numbers of red mycobacteria were observed in every field. They occurred in the margins of residual globi, in many cells, and scattered over the surface of the sectioned tissues.

Restorative Methods

Restorative and rejuvenating methods for "decrepit" mycobacteria in sections have been investigated and reviewed by Wade (3). The sections from one of 6 leprosy patients containing myriads of red bacilli after sulfuric differentiation could be made to show high numbers of red bacilli after differentiation in 0.05 per cent methylene blue in alcohol at pH 6 (1), provided deparaffinization had been conducted in Wade's mineral oil and turpentine. The tissues of one other patient required treatment with turpentine before satisfactory differentiation of the mycobacteria could be achieved, even with the sulfuric reagent.

DISCUSSION

The tissue sections studied in greatest detail were abnormal in regard to the high content and insecure anchorage of the mycobacteria. The mycobacteria themselves probably were atypical because of having been derived from sulfone-treated lepromatous patients. It is necessary, therefore, to distinguish two problems which may arise in dealing with such sections: the loss of mycobacteria and the necessity for differentiating reagents which are more discriminatory than conventional decolorizers.

The conclusion that losses of bacilli explain in part the inferiority of tissue sections for diagnostic purposes has been supported by quantitative counts in sections of human lung involved with miliary tuberculosis. In one respect, these losses should be comparable with the losses of other microorganisms. Like other phagocyted particles, the mycobacteria are apparently not attached to cell structures, but presumably are simply enclosed in the cytoplasm. In thin sections, practically all cells have been sliced open top and bottom. However, the paraffin oil, lipid, and lipid-solvent affinities of the mycobacteria are great (4, 5). These affinities, and the violent mechanical disturbances described, may constitute a special liability for such microorganisms during rapid deparaffinization.

The liberation of mycobacteria from tissue sections by the action of paraffin solvents, and by interaction of alcohol with these solvents and with water, throws light on practices long since found desirable for histopathologic work with leprosy sections. Nearly thirty years ago, Wade recognized advantages in removing paraffin from sections, and water from the stained sections, by the

use of essential oils. For many years Wade and others (6) have avoided the harmful effects of the dehydrating-clearing sequence by drying the sections before mounting a coverglass. A general agreement has developed that deparaffinization in the presence of viscid oils is advantageous (2, 3). The present observations support these concepts and practices on the following basis:

The apparent loss of acid-fastness after applying in succession a series of active solvents is due in part to mechanical losses of bacilli. The improved demonstration of mycobacteria after deparaffinization in the xylene-cottonseed oil mixtures of Fite and associates (2) and in the mineral oil plus aviation gasoline of Wade (3) is due in part to retaining a higher proportion of the bacilli in the sections

These considerations emphasize the importance of employing aqueous rather than alcoholic differentiators. Moreover, these considerations emphasize the contrast between the disruptive effects of acid-alcohol and the fixative properties of carbolfuchsin, sulfuric acid, and the acid sulfates (1). By the use of Gabbett's differentiator, Lie (7) became the first to demonstrate leprosy bacilli in sections from every case of tuberculoid leprosy which he studied. The sulfuric differentiators, or acid sulfates as decolorizing agents, open the possibility that sections may be processed with improved cytology and histology while retaining maximal numbers of mycobacteria.

Separate and distinct from mechanical losses is impaired resistance to alcoholic decolorizers,[3] whether as a result of the species of mycobacterium involved; the physiologic state of mycobacteria in old or resolving lesions; the effects of chemotherapy; or the reagents through which tissue sections are processed. These impairments have been investigated and emphasized by Wade (3).

In respect to sensitivity to alcoholic decolorizers and differentiators, the leprosy bacilli in the sections studied resemble *M. phlei*. Their optimal demonstration is favored in two ways: by deparaffinizing with turpentine or in Wade's turpentine-oil mixtures; and by sulfuric differentiation, as demonstrated here and elsewhere (1).

Recent observations by Lowy and Ridley (8) confirm the mechanical loss of bacilli during rapid deparaffinization and Wade's results with turpentine. The action of turpentine is attributed to terpenes which confer a resistance to acetic acid decolorization but not to the action of alcohol.

[3] This problem can be studied under circumstances which do not permit loss of bacilli. It is necessary to deal only with mycobacteria engulfed by intact cells, in impression films, or in spreads on glass slides. Such films can be exposed to fixatives, impregnated with paraffin, deparaffinized, stained, and differentiated without mechanical loss. From limited observation in cells containing saprophytes, tubercle bacilli, or murine leprosy bacilli, it is evident that removal of paraffin with xylene or benzene reduces the basophilism of both cells and mycobacteria. Nevertheless, if decolorization or differentiation is conducted with weaker solutions, or by appropriately curtailing exposure, the end points of differentiation balance correctly; the pathogenic mycobacteria are stained brilliantly. The clue to the loss of basophilism is the necessity for counterstaining at higher pH or in stronger dye solutions. If, on the other hand, paraffin is removed by heat (Wardekar) or by turpentine-oil mixtures, basophilism is preserved and differentiations proceed normally.

Many of the imponderables in the microscopic diagnosis of mycobacterial disease by use of tissue sections can be obviated by a less expensive, more direct and sensitive method.

The fixative properties of carbolfuchsin and the sulfuric differentiators prevent the loss of tissue cells from phagocytic preparations and impression films. This retention of cells and unmodified bacilli probably explains observations which for a time seemed unorthodox, namely, that, in the experience of the writer, a search for mycobacteria in sections has often failed. Meanwhile, the relationships between the bacilli and host cells had been revealed by direct examination of contact impressions from the cut surfaces of small pieces of fresh tissue.

Such films should be made by pressing the tissue on a series of sites on glass slides *without lateral movement* or smearing. Since the first sites exhibit a preponderance of blood cells and cellular detritus, they may be ignored or made on other surfaces which are discarded. The later ones reveal more clearly the cell-bacillus relationships characteristic of mycobacterial lesions and, therefore, are examined with greatest profit. Pathologic structures described in many types of mycobacterial lesion afford clues concerning useful components to locate with the low-power objective before recourse to the oil immersion. These structures include: macrophages, clusters of cells with expanded or with rosy cytoplasm, cells stuffed with mycobacteria, et cetera. For many purposes histologic observation can be replaced by more adequate development of cytologic interpretation.

SUMMARY

Mycobacteria are lost from paraffin-embedded sections during rapid deparaffinization and also as a result of turbulent interactions between alcohol and solvents and alcohol and water.

Higher proportions of bacilli are retained by slow deparaffinization, by procedures which avoid the use of alcohol, and by decolorizing or differentiating in aqueous reagents.

Sulfuric acid differentiators and acidified sulfates as decolorizers are reagents of choice because of their fixative action and high selectivity in discharging carbolfuchsin from tissue components while leaving it in mycobacteria.

Impression films prepared directly from the cut surfaces of organs may, for many purposes, provide essential information concerning mycobacterial lesions more sensitively and reliably than paraffin sections.

SUMARIO

La Retención y Diferenciación de las Micobacterias en Cortes de Tejidos

En los cortes incrustados en parafina se pierden micobacterias durante la desparafinización rápida y también a consecuencia de interacciones turbulentas entre alcohol y solventes y alcohol y agua.

Retiénense mayores proporciones de bacilos por la desparafinización lenta, por procedimientos que evitan el uso de alcohol y descolorando o diferenciando en reactivos acuosos.

Los diferenciadores de ácido sulfúrico y los sulfatos acidificados como descoloradores

son los reactivos de elección debido a su acción fijadora y alta selectividad despidiendo carbolfuchsina de los componentes histológicos mientras que la dejan en las micobacterias.

Las radiografías de impresión, preparadas directamente de las caras cortadas de los órganos, pueden, para muchos fines, suministrar información esencial acerca de las lesiones micobacterianas con mayor sensibilidad y fidedignidad que los cortes en parafina.

RESUME

La rétention et la différenciation des mycobactéries dans les coupes de tissus

Les mycobactéries disparaissent des coupes paraffinées durant la dé-paraffinisation rapide et aussi à la suite de réactions vives entre l'alcool et les solvants et l'alcool et l'eau.

Des proportions élevées de bacilles sont retenues par le procédé de dé-paraffinisation lente qui évite l'usage de l'alcool ou par les procédés de décoloration ou de différenciation utilisant des réactifs aqueux.

L'acide sulfurique et les sulfates acidifiés comme pour la différenciation comme décolorants sont des réactifs de choix, en raison de leur pouvoir de fixation et de sélection élevée permettant la libération de la carbofuchsine des composants tissulaires sans la déplacer des mycobactéries.

Les repliques préparées directement à partir de la surface des coupes d'organes peuvent, pour des fins diverses, procurer des informations essentielles sur les lésions mycobactériennes plus perceptibles et plus fidèles que celles fournies par les coupes paraffinées.

Acknowledgment

The writer is indebted to Dr. Chapman H. Binford, Armed Forces Institute of Pathology, Washington 25, D. C., for examining this manuscript. Dr. Binford has emphasized earlier the predilection of *M. leprae* for tiny nerves, and pointed out that complete structures cannot be transferred to impression films.

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CULTIVATION OF M. TUBERCULOSIS FROM URINE AND GASTRIC LAVAGE BY THE PANCREATIN-QUATERNARY AMMONIUM COMPOUND METHOD[1]

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INTRODUCTION

Earlier reports have been published on the combined use of proteolytic enzymes and quaternary ammonium compounds for cultivation of tubercle bacilli from sputum specimens by inoculation onto slants (1, 2) and by slide cultivation (3). The application of this method to specimens of urine and gastric lavage is dealt with in the present report.

Some promising results of cultivation of tubercle bacilli from sputum using a quaternary ammonium compound not dealt with in the last report will also be mentioned.

MATERIAL AND METHODS

Specimens: The specimens of urine and gastric washings were selected at random from those received for examination in the laboratory.

Reagents: Pancreatin and methylphenyl-dodecyl-trimethyl-ammonium-methosulfate (Desogen®) have been used as in a previous study (1). A detailed description has been given (2) of the preparation of the reagent (pancreatin-D(1.5 per cent) mixture). The use of Desogen in the final concentration of 1.5 per cent in this mixture, instead of 1 per cent as in the first report (1), may be emphasized.

Procedure: Urine. A specimen was thoroughly shaken and aliquots transferred to two centrifuge tubes (approximately 15 ml.) so that somewhat less than two-thirds of the tubes were filled.

Approximately one-half volume of the pancreatin-D(1.5 per cent) mixture was added to one of the tubes. The tube was stoppered, shaken by hand for a short time, and placed in a dark cupboard at room temperature. On the following morning, that is, after eighteen to twenty-four hours' treatment time, the tube was centrifuged for twenty minutes before inoculation from the sediment.

The second tube was centrifuged for twenty minutes. To the sediment was added an equal volume of a 4 per cent sodium hydroxide solution. After incubation for twenty minutes at 37°C., the tube was centrifuged for twenty minutes. The sediment was neutralized with N HCl using phenol red as indicator.

Gastric lavage. The whole gastric lavage specimen was centrifuged. From the sediment, aliquots were transferred to two test tubes. To one tube was added an equal volume of pancreatin-D(1.5 per cent) mixture. To the other were added two volumes of 4 per cent sodium hydroxide solution.

The remainder of the procedure was the same as for urine except that the portion treated with pancreatin-D(1.5 per cent) was not centrifuged again.

In all cases 1 to 2 drops of the treated specimens were inoculated onto each of six Löwenstein-Jensen slants by means of Pasteur pipettes. The slants, in tubes measuring approximately 13 by 95 mm., were incubated with the surface of the medium approximately horizontal for six weeks and were examined for growth once a week from the third week on.

[1] From the Bacteriological Department, State Institute for Public Health, Oslo, Norway.

In spite of slight differences in the pancreatin-D(1.5 per cent) techniques, the pancreatin D(1.5 per cent) method has been used as a common designation.

RESULTS

The pancreatin-D (1.5 per cent) and the sodium hydroxide methods are compared with respect to numbers of positive cultures of tubercle bacilli and contaminants in tables 1 and 2. The results with urine and gastric lavage specimens are given separately in the tables. The only specimens designated as contaminated were those in which sufficient growth occurred on three or more of the six slants to reduce the chance of obtaining growth of tubercle bacilli.

A larger number of cultures positive for tubercle bacilli was obtained by using the pancreatin-D (1.5 per cent) method than by using the sodium hydroxide method, both in the examination of urine and of gastric lavage specimens. The actual numbers of cultures positive for tubercle bacilli were, respectively, 54 versus 47 and 61 versus 55. The total number of specimens examined consisted of 688 specimens of urine and 659 specimens of gastric washings.

These data may be subjected to statistical analysis, using the sign test of Dixon and Mood (4). First, however, the data which, according to table 1, showed

TABLE 1

DISTRIBUTION OF CULTURES POSITIVE FOR M. TUBERCULOSIS OBTAINED BY CULTIVATION OF URINE AND GASTRIC LAVAGE SPECIMENS BY THE PANCREATIN D (1.5 PER CENT) AND BY THE SODIUM HYDROXIDE METHODS

Nature of Specimens	Noncontaminated Specimens			Contaminated Specimens		Total Numbers of Specimens Positive for *M. Tuberculosis*		
	Both Methods Positive	PD* Positive; NaOH† Negative	PD Negative; NaOH Positive	PD Positive; NaOH Contaminated	PD Contaminated; NaOH Positive	PD	NaOH	Both Methods
Urine	40‡	13	7‡	1	0	54	47	61
Gastric lavage	43	17	12	1	0	61	55	73

* PD = pancreatin-D (1.5 per cent) method.

† NaOH = sodium hydroxide method.

‡ One of these specimens was recorded in this group although three slants inoculated by the NaOH-treated aliquot were contaminated.

TABLE 2

CONTAMINATION RATES OF THE PANCREATIN-D (1.5 PER CENT) AND SODIUM HYDROXIDE METHODS

Nature of Specimens	Numbers and Percentages of Noncontaminated Specimens	Numbers and Percentages of Specimens with at Least 3 of 6 Slants Contaminated				Total
		PD Only		NaOH Only	Both Methods	
Urine	657 (95.49%)	16	(2.33%)	12 (1.74%)	3 (0.44%)	688
Gastric lavage	651 (98.79%)	4	(0.61%)	3 (0.46%)	1 (0.15%)	659

See table 1 for explanation of the abbreviations.

TABLE 3

COMPARISON OF THE GROWTH RATE OF TUBERCLE BACILLI FROM URINE AND GASTRIC LAVAGE AFTER TREATMENT WITH PANCREATIN-D (1.5 PER CENT) AND WITH SODIUM HYDROXIDE

The specimens which were positive by both methods have been arranged according to the time intervals in weeks between the appearance of colonies by the two methods

Methods		PD		Both Methods	NaOH		Total
Antecedence of growth in weeks		2	1	0 = simultaneous growth appearance	1	2	
Distribution of specimens	Urine	2	7	31	0	0	40
	Gastric lavage	5	5	26	6	1	43

See table 1 for explanation of the abbreviations.

positive and negative results on both methods were deleted from the analysis. Statistically significant differences were not found between the two methods in either case.

The over-all contamination rates for urine specimens were 2.77 per cent by the pancreatin-D (1.5 per cent) method against 2.18 per cent by the sodium hydroxide method (table 2). The corresponding figures for gastric lavage specimens were 0.76 per cent and 0.61 per cent, respectively.

The actual numbers of table 2 have been subjected to statistical analysis in the manner described above. No significant differences between the two methods with respect to contamination were found.

It may be noted in table 1 that one specimen of urine as well as one of gastric lavage were found positive for tubercle bacilli by the pancreatin-D (1.5 per cent) method, but they were found negative and contaminated by the sodium hydroxide method. It seems reasonable to assume that this contamination has prevented a positive result by the sodium hydroxide method. No similar apparent loss of a positive result for tubercle bacilli was found to be due to contamination with the pancreatin-D (1.5 per cent) method.

Two specimens yielded tubercle bacilli by the sodium hydroxide method although in both instances three of the six slants used were contaminated (table 1).

In table 3 it may be seen that with both kinds of specimens the growth of tubercle bacilli frequently appeared earlier with the pancreatin-D (1.5 per cent) method than with the sodium hydroxide method.

Since the last report (2) has appeared, investigations dealing with the merits of quaternary ammonium compounds in the cultivation of tubercle bacilli have proceeded. Promising results have thus been obtained with Vantoc B[3] which is based essentially on tetradecyl pyridinium bromide. Replacement of Desogen by Vantoc B led to superior results with sputum specimens. These favorable

[3] A product of Imperial Chemical Industries Limited, England.

results were found with respect to both positive results and contamination rates of the specimens. It may be pointed out that the differences found between the results of the two agents were not statistically significant.

DISCUSSION

The same superiority of the pancreatin-D (1.5 per cent) over the sodium hydroxide method seems to exist regardless of the nature of the specimens as far as the studies have been carried out. In addition to the urine and gastric lavage specimens tested in the present study, the sputum specimens of the previous report (2) are of interest in this connection.

In cultivation of tubercle bacilli from these three types of specimens, the pancreatin-D (1.5 per cent) method tended to be superior to the sodium hydroxide method. This consistent tendency with different kinds of specimens gives more weight to the view that the pancreatin-D (1.5 per cent) method may be superior to the sodium hydroxide method than would be accomplished by considering each individual kind of specimen separately.

The same superiority of the pancreatin-D (1.5 per cent) method prevails in matching the two methods with respect to tendency to give the earlier appearance of growth of tubercle bacilli. The results of the previous report (2) concerning sputum specimens also contribute largely to maintain this view. It may be pointed out that this tendency in favor of the pancreatin-D (1.5 per cent) method seems marked.

On the other hand, the sodium hydroxide method seems to give a slightly less degree of contamination than the pancreatin-D (1.5 per cent) method. This appears to be valid for all kinds of specimens so far tested.

It may be pointed out that the degree of contamination with both methods is slight. Only a few specimens positive for tubercle bacilli are lost due to contamination; in this study only two specimens were lost with the sodium hydroxide method, and none with the pancreatin-D (1.5 per cent) method. However, it is unknown whether any of the specimens contaminated by both methods contained tubercle bacilli. Yet the total number of such contaminated specimens is low and, in relation to the frequency of specimens containing tubercle bacilli, it is probable that the number of positive cultures lost may be very small or none at all.

The main point concerning cultivation of tubercle bacilli is to obtain a maximal number of positive results. Nevertheless, in connection with the aspects of contamination discussed above, the apparently favorable decontaminating effect obtained with Vantoc B reported earlier in this article may be recalled. As stated, the strong decontaminating effect of Vantoc B did not seem to reduce the number of positive cultures.

Somewhat different techniques have been used for different types of specimens. This applies both for the procedure with the pancreatin-D (1.5 per cent) mixture and with the sodium hydroxide solution. These technical variations are arbitrary adjustments to particular experimental conditions established by the

different types of specimens. It is uncertain whether anything is gained by maintaining these technical modifications, and extensive work would be necessary to decide upon the optimal technique for each kind of specimen.

Some of the reasons for the choice of these technical variations may be mentioned. Smaller volumes of pancreatin-D (1.5 per cent) and of sodium hydroxide reagents were used in the treatments of urines, since homogenization is superfluous and the urines frequently are contaminated to a lesser degree than sputum. Furthermore, unnecessary dilution of the sample is thereby avoided.

The question whether the addition of pancreatin to the urine specimens is at all advantageous needs further study. In this work the same reagent was used as for sputum and gastric lavage specimens for reasons of convenience. Since gastric lavage sediments frequently are of a consistency similar to sputum, the same treatment technique was used in both cases.

It may be of interest to test whether or not the decrease of the surface tension brought about by the presence of the surface active agent may provide better concentration of the tubercle bacilli during centrifugation. Centrifugation did, however, not seem to increase the sensitivity of the pancreatin-D method applied to sputum specimens, as shown in a previous report (3); but this study was not extensive.

In the present study, as also in the previous reports, small culture tubes have been used due to insufficiency of incubator space. Culture tubes allowing for larger quantities of medium might be better suited for the pancreatin-D method, since the quaternary ammonium compound might be neutralized more efficiently and the pancreatin effect reduced by dilution.

In this connection reference may be made to some experiments as yet unpublished on the effect of the size of the inoculum. Whereas inocula of 1 or 3 drops of sputum specimens treated with pancreatin-D gave approximately equally good results, an increase to 10 drops led to considerable lessening of efficiency, both as to positive results and contamination. Furthermore, marked softening of the medium was apparent. The pancreatin-D mixture was of final concentrations of both agents of 1 per cent. It should be noted further that the pancreatin used, the same as in all these reports, had a very strong proteolytic activity and that the same small culture tubes were used.

The slants should be incubated with the surfaces of the medium approximately horizontal at least for one or two days. This allows the inoculum to soak into the whole surface of the medium. No matter what method is used for pretreatment, this point may seem of importance, but an additional question arises when the pancreatin-D method is concerned. Thus, to place the tubes vertically immediately after inoculation leads to accumulation of greater parts of the inocula at the bottom of the tubes. This may reduce to a certain degree the neutralization of the germicidal action of the quaternary ammonium compound, and it further leads to a great local action of proteolytic enzyme.

The saving of work is considerable by using the pancreatin-D (1.5 per cent) method.

The statistical sign test of Dixon and Mood (4) fits the present experimental

conditions well. These conditions vary from one specimen to another; but this variation is part of the assumptions underlying the test. Deleting from consideration the extreme results, viz., concordant results by both methods, gives a more sensitive test.

SUMMARY

The combined use of pancreatin and methylphenyl-dodecyl-trimethyl-ammonium-methosulfate (Desogen®) compares favorably with the use of sodium hydroxide in cultivating urine and gastric lavage specimens for tubercle bacilli. This holds true when both results and ease of work are considered. Mention is made of favorable results obtained with sputum specimens using a procedure in which Vantoc B®, essentially based on tetradecyl pyridinium bromide, was used in mixture with pancreatin.

SUMARIO

Cultivo del Mycobacterium Tuberculosis Procedente de la Orina y del Lavado Gástrico en la Pancreatina y el Compuesto Cuaternario de Amonio

El empleo combinado de la pancreatina y del metilfenil-dodecil-trimetil-amonio metosulfato (Desogén®) se compara favorablemente con el uso del hidróxido de sodio para el cultivo de ejemplares de orina y de lavados gástricos en busca de bacilos tuberculosos. Esto es un hecho cuando se consideran tanto los resultados como la facilidad de la tarea. Menciónanse los resultados favorables obtenidos en ejemplares de esputo usando un procedimiento en que se empleó, mezclado con la pancreatina, Vantoc B®, basado esencialmente en el bromuro tetradecílico de piridinio.

RESUME

Culture du Mycobactérium tuberculosis à partir de l'urine et des liquides de lavage gastrique par la méthode du composé ammonium quaternaire pancréatine

L'usage combiné de pancréatine et de méthosulfate de méthylphényl-dodécyl-triméthyl-ammonium (Desogèn®) soutient favorablement la comparaison avec l'usage d'hydroxyde de sodium dans la culture des prélèvements d'urine et de lavage gastrique pour la recherche du B. K. Ceci se vérifie quand on considère les résultats et la facilité du procédé. L'auteur mentionne les résultats favorables obtenus avec les échantillons de crachats lorsque le procédé au Vantoc B®, qui est essentiellement basé sur le bromure de tétradécyl pyridinium, était employé en lui associant la pancréatine.

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Case Report

SCROTAL PNEUMOCELE COMPLICATING PNEUMOPERITONEUM[1]

JOHN J. WALSH, RICHARD H. LINN, AND WILLIAM H. SAGE, III

(Received for publication March 26, 1956)

The literature contains approximately ten cases of scrotal pneumocele complicating pneumoperitoneum (1). The following case is reported because of the rarity of the condition and the recurrence of this complication after four additional months of pneumoperitoneum. This latter phenomenon has not previously been reported.

CASE REPORT

A 26-year-old male was hospitalized on September 10, 1951, because of cough of one month's duration. He gave a history of intimate contact with a known tuberculous patient and admitted a 30-pound weight loss in the preceding six months. Physical examination revealed a thin male who appeared acutely and chronically ill. With the exception of bilateral post-tussic apical rales no other abnormalities were noted. A roentgenogram of the chest showed bilateral fibrocaseous lesions with a 3 cm. cavity in the right upper lung posteriorly. The sputum contained tubercle bacilli on smear and culture.

In addition to the usual symptomatic and supportive therapy, the patient was given daily PAS and streptomycin in the usual dosages. After four weeks, the streptomycin was reduced to a twice-weekly schedule.

On November 1, 1951, pneumoperitoneum was initiated and maintained without difficulty until the first days of December, 1951. During this entire period the patient complained of vague lower abdominal distress of a cramping nature. This was never of severity sufficient to require treatment nor was it associated with abnormal physical findings. Once, on November 21, 1951, after a refill, the patient developed mild transitory subcutaneous emphysema of the right upper abdominal and lower thoracic wall.

Late in the night of December 12, 1951, the patient developed an increase in the severity of the abdominal cramps which soon became constant and localized to both lower quadrants. By the following morning a low-grade fever and leukocytosis had appeared. Because of the possibility of appendicitis an exploratory laparotomy was performed that same morning. A ruptured appendix was removed and the patient enjoyed an uneventful recovery. Because of the discovery of tubercle bacilli in the surgical specimen the patient again was given the streptomycin daily with continued clinical and roentgenographic improvement.

Pneumoperitoneum was reinstituted on March 6, 1952, with 600 cc. of air introduced into the peritoneal cavity. An additional 600 cc. were given two days later. Within three hours the patient developed pain and slight fullness of the left side of the scrotum with crepitation only on palpation of the left spermatic cord. The patient was treated with analgesics and a suspensory. During the next twenty-four hours the pain and tenderness disappeared although the fullness and crepitation persisted for an additional forty-eight hours. On March 13, 1952, 400 cc. of air were given without difficulty. That evening there was a recurrence of scrotal pain with crepitation and tenderness of the spermatic cord. These symptoms were milder than those of the previous episode and, with the exception of crepitation, disappeared within twenty-four hours. The crepitation persisted an additional twenty-four hours. Subsequently a satisfactory pneumoperitoneum was maintained with weekly refills of approximately 1,000 cc. There were no difficulties until July 10, 1952, when, because of

[1] United States Public Health Service Hospital, New Orleans, Louisiana.

the abrupt onset of abdominal cramps and discomfort, the procedure was discontinued after only 500 cc. had been introduced. That evening the patient developed moderately severe left testicular pain, visible fullness of the left side of the scrotum, and tenderness and crepitation of the spermatic cord. Analgesics and a suspensory were provided. Within three days the swelling and crepitation had disappeared although the patient continued to complain of a dull ache in the left testicle and abdominal cramps for approximately one week. Pneumoperitoneum was abandoned. Subsequently the patient continued to do well. He later left the hospital to seek institutionalization closer to his home and was lost to follow-up.

COMMENT

It is generally accepted that the appearance of a scrotal pneumocele during pneumoperitoneum therapy is dependent upon the presence of a patent processus vaginalis. This complication usually appears early in the course of pneumoperitoneum and promptly disappears. Once cleared, it has not previously been reported to recur, despite continuation of collapse therapy. The continuous irritation of increased intra-abdominal pressure is thought to lead to closure of the patent processus vaginalis.

The abrupt appearance of lower abdominal pain and the recurrence of the pneumocele during a refill, but after four additional months of pneumoperitoneum, suggests that the processus had incompletely closed, only to be reopened when the intra-abdominal pressure was sufficiently elevated.

SUMMARY

A case of scrotal pneumocele complicating pneumoperitoneum is reported. The case is unique in that this complication recurred after four additional months of collapse therapy. Moreover, while maintaining pneumoperitoneum, the patient developed acute appendicitis, for which he was successfully treated.

SUMARIO

Neumocele Escrotal como Complicación del Neumoperitoneo

Preséntase un caso de neumocele escrotal que complicó un neumoperitoneo. Es único en su género por haber recurrido esta complicación al cabo de cuatro meses más de colapsoterapia. Además, mientras mantenía el neumoperitoneo, el enfermo manifestó apendicitis aguda, que fué tratada con éxito.

RESUME

Pneumocèle scrotal complication d'un pneumopéritoine

Les auteurs rapportent un cas de pneumocèle scrotal secondaire à un pneumopéritoine. Ce cas est unique en ce sens que la complication a récidivé au bout de quatre mois additionnels de collapsothérapie. En outre, pendant l'entretien du pneumopéritoine, le malade a eu une crise d'appendicite aiguë qui a été traitée avec succès.

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PERIARTERITIS-LIKE ILLNESS[1]

Report of a Case of Pulmonary Cavitation

LOUIS ZASLY AND GEORGE L. BAUM

(Received for publication November 2, 1955)

INTRODUCTION

The purpose of this report is to present a case of an illness involving multiple systems with unusual pulmonary roentgenographic findings and with a prolonged remission incident to hormonal therapy.

CASE REPORT

N. L., a 30-year-old white male postal clerk, was first admitted to this hospital on December 5, 1951. For five months prior to admission, he had intermittent fever, weight loss, productive cough, and occasional hemoptysis. During this time he received sulfonamides, penicillin, streptomycin, chloramphenicol, and chlortetracycline without benefit. Six weeks prior to admission he began to have intermittent vomiting after meals.

The past history and system review were noncontributory. The patient's mother was stated to have hay fever and his maternal grandmother was believed to have had "asthma."

On admission to the hospital the patient was acutely ill. The temperature was 101° F., orally; the pulse was 104 per minute; the respirations were 28 per minute. The blood pressure was 150 systolic, 90 diastolic in mm. of mercury. Respirations were labored and diaphragmatic motion was restricted. Expiration was prolonged and the breath sounds were distant. An old, well-healed inguinal herniorrhaphy scar was noted. The muscles of the extremities were flabby. Bilateral pes cavus was present. The deep tendon reflexes of the right upper and lower extremities were overactive. There was bilateral ankle clonus. Atrophy of the right triceps and biceps and of the interossei of both hands was present.

Initial laboratory studies revealed 4-plus albuminuria with many hyaline and granular casts and microscopic hematuria. The leukocyte count was 30,100 cu. mm. with 41 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes, 48 per cent eosinophils, and one per cent basophils. The erythrocyte count was 2,900,000 per cu. mm.; hemoglobin, 7.5 gm. The erythrocyte sedimentation rate was 28 mm. (Wintrobe, corrected). The sputum contained numerous leukocytes and *Streptococcus viridans* was obtained on culture. No acid-fast bacilli were detected in the sputum. Three blood cultures were sterile. Total serum proteins were 7.3 gm. per 100 ml., albumin 3.8 gm., and globulin 3.5 gm. per 100 ml. Blood urea nitrogen was 32 mg. per 100 ml. A phenosulfonphthalein test revealed 5 per cent excretion in fifteen minutes.

A roentgenogram of the chest obtained on December 7, 1951, revealed slight increase in the markings in the right first anterior interspace (figure 1). Fluoroscopy of the chest showed paradoxical motion of both leaves of the diaphragm. Diaphragmatic movements were greatly restricted.

On December 11, ventilatory tests were performed according to methods described elsewhere (1). These revealed a vital capacity of 1,200 cc. (33 per cent of predicted normal) and a maximal breathing capacity of 32 liters per minute (25 per cent of predicted normal).

Bronchoscopy, with mucosal biopsy and gastrocnemius muscle biopsy, was nonrevealing. Tissue obtained by liver biopsy showed no abnormalities. Repeated examinations of the stools were negative for ova and parasites. An electrocardiogram showed sinus tachycardia.

The patient was treated symptomatically with a nebulized bronchodilator,[2] saturated

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[1] From the Medical Service, Veterans Administration Hospital, Coral Gables, Florida.

[2] Aerolome compound solution (Lilly) with Zephiran® hydrochloride (aqueous 1:1,000) in equal parts.

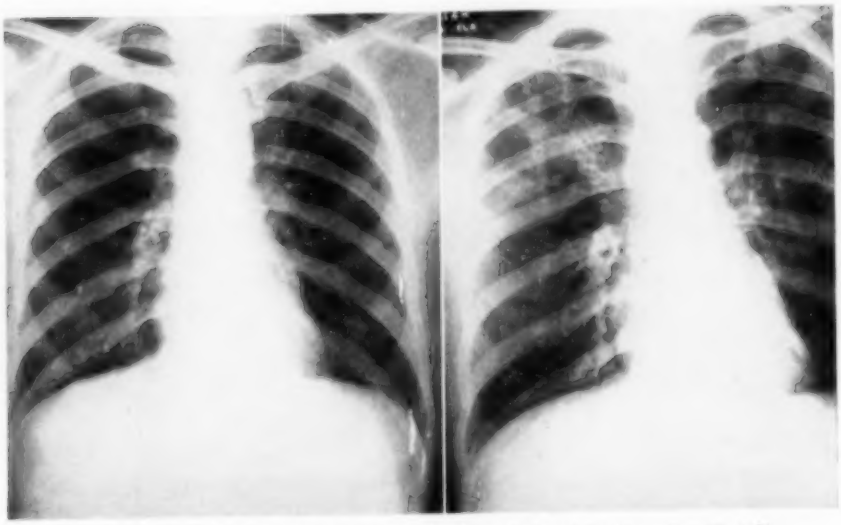

FIG. 1 (Left). Initial roentgenogram of the chest obtained on December 17, 1951.
FIG. 2 (Right). Roentgenogram obtained on February 4, 1952, showing soft infiltrate in right upper lobe.

potassium iodide solution orally, and aminophylline intravenously and rectally, with little change. A course of intramuscular cortisone was started on December 21, consisting of 300 mg. the first day, 200 mg. the second day, and 100 mg. daily thereafter. The symptoms quickly subsided, together with disappearance of the eosinophilia. There was improvement in the urinary findings. Vital capacity on January 2, 1952, was 3,300 cc., or 92 per cent of predicted vital capacity; and the maximal breathing capacity was 90 liters per minute, or 69 per cent of predicted capacity.

When cortisone was discontinued on January 16, 1952, symptoms returned. The drug was resumed on January 25, with prompt improvement. Ten days later the patient developed a cough productive of yellow sputum which cultured hemolytic *Staphylococcus aureus*. A chest roentgenogram on February 4 revealed a soft infiltrate in the right upper lobe with some increase in the markings in the left anterior interspace (figure 2). Cortisone therapy was discontinued. Chlortetracycline, 2 gm. daily, was given for three weeks beginning February 7, 1952. During this interval, fever, dyspnea, and productive cough were present. A cavity in the right second anterior interspace was demonstrable on February 15 (figure 3). This increased in size with the formation of a fluid level by March 3 (figure 4).

Twenty sputum specimens collected up to May 1 were negative for acid-fast bacilli on microscopy and culture. The tuberculin reaction of the skin to purified protein derivative (first-strength) was reported positive on March 5. Sputum was repeatedly negative for the larger parasites.

Streptomycin, 1 gm. intramuscularly, was given daily from February 28 to March 20, 1952, when it was discontinued because of severe vertigo. Para-aminosalicylic acid (PAS), 12 gm. orally, was administered daily from February 28 to June 4. After four days of this therapy the patient was afebrile, but cough, blood-streaked sputum, wheezing, tachycardia, anemia, and eosinophilia persisted. Bronchodilators were frequently required. Fever returned April 13, and four days later a fresh infiltrate was detected in the left upper lobe (figure 5). The right upper lobe infiltrate persisted with a thin-walled area of radiolucency overlying the right second rib anteriorly.

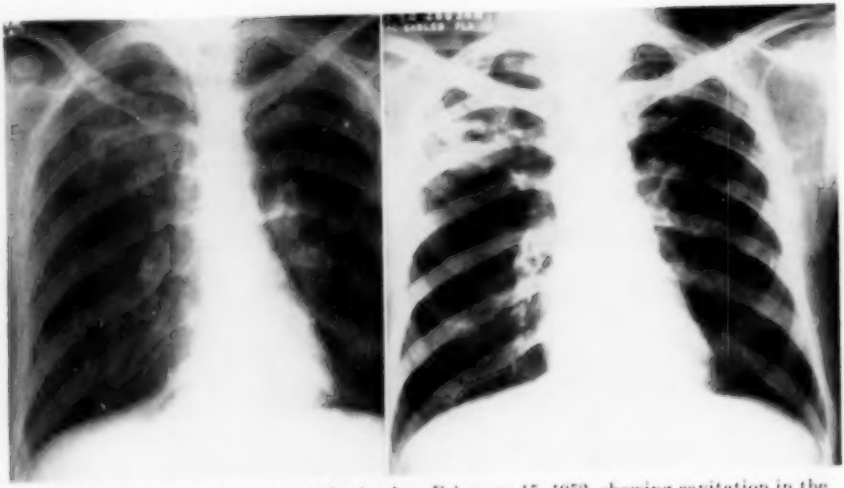

FIG. 3 (Left). Roentgenogram obtained on February 15, 1952, showing cavitation in the right second anterior interspace.

FIG. 4 (Right). Roentgenogram obtained on March 3, 1952, showing cavity filled with fluid level after chlortetracycline and oxytetracycline therapy.

FIG. 5 (Left). Roentgenogram obtained on April 17, 1952, showing fresh infiltrate in left first and anterior interspaces appearing while on PAS therapy.

FIG. 6 (Right). Roentgenogram obtained on June 3, 1952, showing improvement after corticotropin-cortisone combined with dihydrostreptomycin and PAS. Cystic area in right first anterior interspace.

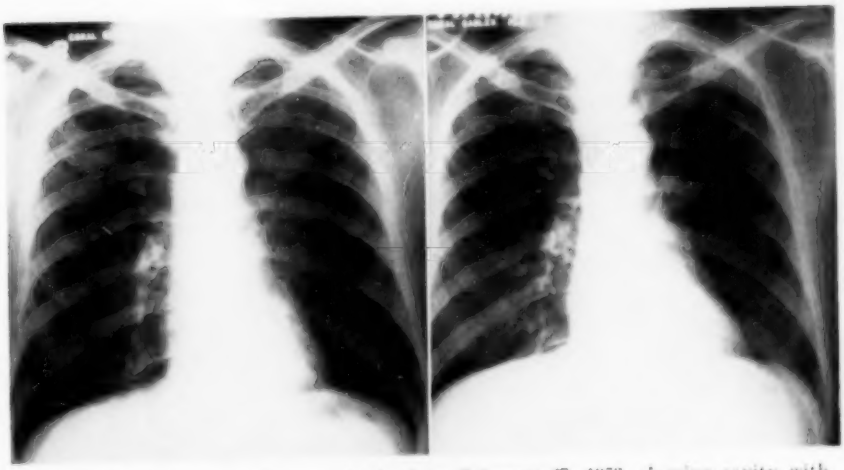

FIG. 7 (Left). Roentgenogram obtained on February 27, 1953, showing cavity with fluid level in R.U.L. On maintenance cortisone therapy.

FIG. 8 (Right). Roentgenogram obtained on March 9, 1954, showing definite improvement after corticotropin therapy.

Corticotropin, 20 mg. daily by slow intravenous drip, was administered from April 18 for eleven days, followed by cortisone orally, 100 mg. per day. The PAS administration was maintained. Dihydrostreptomycin, 1 gm. daily, was started on April 18 as a prophylactic measure. The patient became asymptomatic in three days. Albuminuria and eosinophilia again diminished. By June 3, 1952, there was considerable improvement in the appearance of both upper lobes (figure 6). A cystic area in the right infraclavicular region was noted.

Repeated examinations of sputum and gastric washings continued to be negative for acid fast bacilli and fungi on microscopy and culture. A guinea pig inoculated from a seven-day sputum collection on March 24 was sacrificed on June 27 and found to be free of tuberculosis.

The patient was discharged, asymptomatic, on June 24, to continue on oral cortisone, 100 mg. per day, a low salt intake, and potassium chloride, 6 gm. per day. He was re-admitted on four occasions during the next five months for episodes of dyspnea, wheezing, and cough. Intravenous corticotropin and bronchodilators were successfully employed during these admissions. Oral cortisone was given between the courses of corticotropin. Roentgenograms of the chest continued to show improvement.

The patient's sixth admission, occasioned by similar symptomatology, was on February 1, 1953. Roentgenograms of the chest on February 2 and February 17 showed no change from those of the previous admission. Intravenous corticotropin was given for nine days before resuming cortisone orally. There was symptomatic improvement. He was given a leave of absence on February 12, returning two weeks later with increased cough, wheezing, and vomiting. A roentgenogram of the chest on February 26 again demonstrated a cavity with fluid level in the right upper lobe (figure 7).

Corticotropin therapy, in divided doses up to 60 mg. per day by the intramuscular route, was next employed, followed by clinical improvement. By March 9, the right upper lobe cavity could not be demonstrated (figure 8). After a few trial home visits on corticotropin gel,[3] the patient was finally discharged on May 19 on 25 mg. per day intramuscularly. He was instructed to remain on a salt-free diet and to take potassium chloride, 6 mg. per day.

[3] Acthar gel (cortisone hormone), Armour.

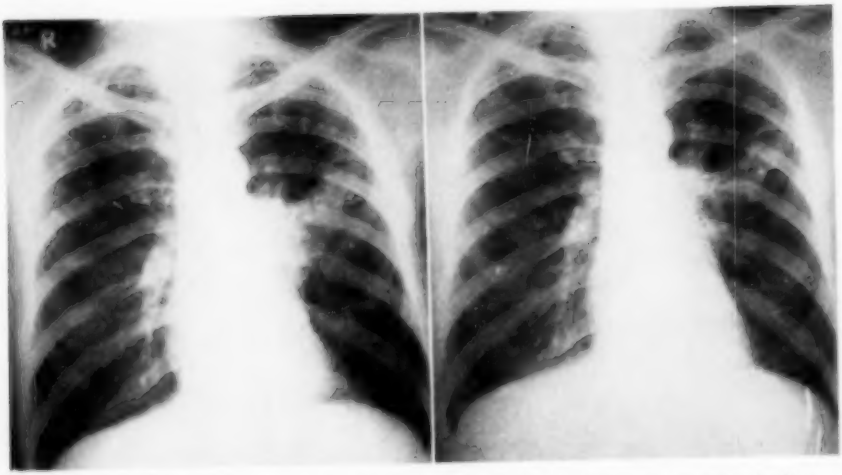

FIG. 9 (Left). Roentgenogram obtained on February 11, 1954, showing irregular infiltration extending from the left hilus.

FIG. 10 (Right). Roentgenogram obtained on February 19, 1954, showing decreased infiltration after increased cortisone dosage.

The patient was well maintained on this therapy until August 21, 1953, when he was hospitalized elsewhere for a genitourinary infection. Chlortetracycline therapy cleared up this process. An attempt at discontinuing hormonal therapy was unsuccessful, and cortisone was resumed after one month, with abatement of wheezing, cough, dyspnea, and eosinophilia. Sputum examinations were repeatedly negative for acid-fast bacilli. The chest roentgenograms showed no change. The patient was discharged on September 29 on a daily dose of 75 mg. of cortisone, later reduced to 50 mg. per day. He did not do as well on this regimen, requiring a bronchodilator spray frequently.

On February 11, 1954, the patient reported to the Veterans Administration Hospital, Coral Gables, Florida, for a follow-up examination. He was not acutely ill and his temperature was 98.6°F. orally. There was cough productive of about one-half cupful of white nonfoul sputum daily. A roentgenogram of the thorax demonstrated an irregular infiltration extending from the left hilus (figure 9). The dosage of cortisone was increased to 100 mg. per day and bronchodilators were prescribed. One week later there was some resolution of the process in the left lung (figure 10). A three-day sputum specimen was negative for acid-fast bacilli on microscopy and culture.

The patient was again hospitalized elsewhere on March 18 for increased cough and expectoration with low-grade fever. A roentgenogram of the thorax revealed a diffuse bilateral linear and reticular infiltration (figure 11). Cortisone therapy was maintained. After a week of therapy with chlortetracycline and erythromycin the temperature returned to normal, but the cough persisted and the diffuse infiltrations appeared to be more extensive (figure 12). Bronchodilators were frequently required. Fever returned three weeks later and persisted until steroid therapy was changed to corticotropin (May 21). At this point the patient became asymptomatic quite dramatically. A left inguinal hernia was repaired on June 9. Marked clearing of the diffuse pulmonary infiltrations was finally noted by June 23 (figure 13). The patient was discharged on July 2, 1954, on corticotropin-gel, 25 mg. intramuscularly per day.

Except for occasional mild wheezing, he did very well until January 10, 1955, when he

FIG. 11 (Left). Roentgenogram obtained on March 22, 1954, showing diffuse bilateral infiltration. On cortisone therapy.
FIG. 12 (Center). Roentgenogram obtained on March 30, 1954, showing diffuse infiltration more extensive. Cortisone therapy.
FIG. 13 (Right). Roentgenogram obtained on June 13, 1954, showing marked clearing of the diffuse infiltrations on cortisone therapy. Cystic area in R.U.L. still present.

was again admitted to the Coral Gables Veterans Hospital. Dyspnea and cough were the presenting complaints. Treatment included readjustment of the corticotropin dosage and the administration of antimicrobials. Roentgenograms of the thorax showed no change from the film of June 23, 1954. The vital capacity was 3,000 cc., or 81 per cent of predicted normal. The maximal breathing capacity was 50 liters per minute, or 69 per cent of predicted normal. A three-second vital capacity was 2,520 cc., or 69 per cent of predicted normal vital capacity. Psychometric testing indicated evidence of organic cerebral damage. The patient was discharged on March 4, 1955, to continue to receive corticotropin and 500 mg. of tetracycline daily and bronchodilators as required.

He did well on this regimen. When prednisone[4] became available, this was substituted. At present the patient is doing very well on 15 mg. of prednisone per day. A bronchodilator spray is only occasionally required. A recent film of the chest (obtained on August 1, 1955) showed no significant change from the film taken on June 23, 1954. Hypertension and albuminuria are still present.

COMMENT

The tentative diagnosis of periarteritis nodosa in the present case was based on the multiple symptomatology incriminating various systems and organs. Thus, the central and peripheral nervous systems, the gastrointestinal tract, the lungs, and the kidneys were involved. There were protracted fever, leukocytosis, eosinophilia, dyspnea with wheezing, cough with expectoration, hypertension, neurologic findings, and albuminuria. The term "periarteritis-like illness" is used because of the absence of histologic proof. Although steroid therapy could not be discontinued, this patient is now doing well four years after the onset of his illness. Evidences of persistent damage include reduction in pulmonary ventilatory function, neurologic and psychometric impairment, persistent hypertension, and albuminuria.

The etiologic aspects, pathogenesis, pathology, and symptomatology of periarteritis nodosa have been well covered in the literature (2–6). The pulmonary lesions have been stated by Zeek (6) to be confined to the bronchial arteries, while Sweeney and Baggenstoss (7) have reported coexistence of bronchial and pulmonary arteriolar involvement in eight necropsied cases. Symptomatology referable to the lungs is quite frequent (8–10). Cough, dyspnea, expectoration, hemoptyses, and "asthmatic" wheezing have been described.

The roentgenographic appearance of the thorax in periarteritis nodosa may vary considerably. Doub and associates (8) reported hilar enlargement, perihilar infiltration, nodular infiltrations with vascular striations, and patchy infiltrative lesions. Ellman and Cudkowicz (9) found that miliary lesions of the upper lobes predominate. In 1950, Sandler, Matthews, and Bernstein (10) reported the first case in which a roentgenographically demonstrable pulmonary cavity was proved at necropsy to be secondary to periarteritis nodosa.

Prior to the advent of hormonal therapy the prognosis had been very poor. The duration of the disease had been from a few months to one year (5). It has been reported that prolonged remissions occur only when corticotropin, cortisone, or related products are maintained (11). Doub and his co-workers (8) found only transient benefit as a result of cortisone therapy in patients with pulmonary involvement. Remissions of more than one year after discontinuance of cortisone or corticotropin have been reported (11–14). Residual vascular damage, particularly in the kidney, has been described as occurring after steroid therapy (11).

The demonstration in this case of recurring pulmonary infiltrations, including cavita-

[4] Meticortin (Schering).

tions in the right upper lobe, over a two-year period is of considerable interest. At the time the infiltration first appeared, *Staphylococcus aureus* was isolated from the sputum, which was yellow. This brings up the possibility of bacterial pneumonitis at that particular time. The failure to respond favorably to antituberculous chemotherapy and the absence of acid-fast bacilli from the sputum are strong evidence against a tuberculous etiology of the lesions. Likewise, the failure to find fungi, despite repeated studies of the sputum, constitutes evidence against mycotic origin of these infiltrates and cavitations.

These abnormal roentgenographic shadows were usually present during periods of increased symptomatology. The shadows were favorably influenced only by adequate doses of cortisone or corticotropin therapy. The latter appeared to be more effective in suppressing the manifestations of the patient's illness.

The possibility remains that these cavitations were related to pulmonary infection and abscess formation. The initial cavity appeared during cortisone therapy, subsequently clearing on antimicrobial and steroid administration. However, the cavity appearing in February, 1953, was no longer demonstrable after ten days of corticotropin intramuscularly as the sole therapy.

Evans and Steinberg (15) have described pulmonary infiltration with cavitation in a leukemic patient treated with cortisone. It was postulated that necrotic infarction had occurred secondary to diminution of reparative processes and increased blood coagulability incident to cortisone administration.

It is believed that the pulmonary cavitation in the present case followed tissue necrosis secondary to thrombosis of larger arteries. The cystic area in the right infraclavicular region was not demonstrated on the initial roentgenogram. It is believed that this represents a local area of emphysema secondary to healing processes.

SUMMARY

A case of periarteritis-like illness is reported. Recurring pulmonary infiltrations and recurring right upper lobe cavitation were demonstrated. These roentgenographic manifestations are believed to represent the effects of vascular damage in the lungs. This case also represents an instance of prolonged survival of a periarteritis-like illness following steroid therapy.

SUMARIO

Enfermedad Parecida a Periarteritis: Presentación de un Caso con Cavitación Pulmonar

Comunícase un caso de enfermedad parecida a periarteritis, en el que se observaron además infiltraciones pulmonares recurrentes y cavernas recurrentes en el lóbulo superior derecho. Estas manifestaciones radiográficas parecen representar los efectos de lesiones vasculares en los pulmones. Este caso ofrece además un ejemplo de sobrevivencia prolongada con la esteroidoterapia.

RESUME

Affection du type périartérite. Rapport sur un cas avec caverne pulmonaire

Observation d'un cas de maladie du type périartérite. Les récidives d'infiltrations pulmonaires et de lésions excavées récidivantes du lobe supérieur droit sont démontrées. Les signes radiologiques sont considérés comme le résultat d'altérations vasculaires dans les poumons. Cette observation montre également un cas de survie prolongée dû à la stéroidothérapie.

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Notes

SOME OBSERVATIONS ON THE PATHOLOGY OF THE LESIONS CAUSED BY ISONIAZID-RESISTANT TUBERCLE BACILLI IN THE GUINEA PIG

After the initial controversy following the article by Middlebrook and Cohn,[1] it is now generally accepted that isoniazid-resistant tubercle bacilli have a reduced virulence for the guinea pig. The degree of attenuation of isoniazid-resistant tubercle bacilli seems to be still a matter of controversy. Rist, Grumbach, and Libermann[2] found some resistant strains which had retained their virulence for guinea pigs, and this observation has been confirmed by Conalty and Gaffney[3] who also described two isoniazid-resistant strains which killed the guinea pig after subcutaneous inoculation.

Most investigators have based their opinion on the course of the disease in laboratory animals experimentally infected with isoniazid-resistant tubercle bacilli, as well as on macroscopic changes in animals which had died or had been killed after a certain period. Only a few investigators have also subjected the organs of the guinea pigs infected with tubercle bacilli to a histologic examination.

Karlson and Ikemi[4] examined 10 guinea pigs killed eighty-eight days after they had been infected. Microscopically, only a few animals were found to have residual foci in the liver, only 2 guinea pigs having well-defined small tubercles in the spleen and the lungs. Macroscopically, all of the animals showed active progressive lesions, which were restricted to the place of injection and the regional lymph nodes.

Barnett, Bushby, and Mitchison[5] infected guinea pigs with isoniazid-resistant tubercle bacilli (H37Rv) and after forty-two days found small accumulations of 10 to 100 histiocytes infiltrated by lymphocytes in the livers of the animals, a smaller number of the same lesions being perceived in the spleens and lungs. After sixty-three days they could find no lesions, or only very small ones (up to 20 histiocytes).

Mitchison,[6] after having injected his laboratory animals with resistant bacilli isolated from patients treated with isoniazid, found that the microscopic examination of the livers, spleens, and lungs, which macroscopically in most instances showed no change, often revealed small tubercles or accumulations of histiocytes and lymphocytes which tended to heal.

The following is a report of an examination of 40 cultures of isoniazid-resistant tubercle bacilli, isolated from patients treated with this drug.

The strains of tubercle bacilli were isolated from the patients on Löwenstein medium with glycerol and without isoniazid. Suspensions of strains resistant to 10 γ of isoniazid per ml. were shaken with beads. After two passages on media with the same concentration of isoniazid the strains were harvested and were injected subcutaneously into the thighs of the guinea pigs in amounts of 1 mg. (moist weight).

As a rule the guinea pigs were killed eight weeks after the infection, the organs being examined both by macroscopic and microscopic methods (fixation in 20 per cent formol, staining with hematoxylin-eosin, hematoxylin—van Gieson, and by the Ziehl-Neelsen method).

The results of the macroscopic and microscopic examination of 40 experimentally infected guinea pigs are set forth in tables 1 and 2.

[1] Middlebrook, G., and Cohn, M. L.: Science, 1953, *118*, 297.
[2] Rist, N., *et al.*: Gaz. méd. France, 1954, *61*, 191.
[3] Conalty, M. L., and Gaffney, E. F.: Am. Rev. Tubere., 1955, *71*, 709.
[4] Karlson, A. G., and Ikemi, Y.: Proc. Staff Meet., Mayo Clin., 1954, *29*, 119.
[5] Barnett, M., *et al.*: Brit. J. Path., 1953, *34*, 568.
[6] Mitchison, D. A.: Brit. M. J., 1954, No. 4854, 128.

TABLE 1

EXAMINATION OF 40 ISONIAZID-RESISTANT STRAINS ON VIRULENCE IN GUINEA PIGS

Macroscopic Lesions at Autopsy of the Guinea Pigs Eight Weeks After Infection

Site of Injection + Regional and/or Lumbo-Sacral Lymph Nodes	Spleen	Liver	Total Number
+	+	+	13
+	+	−	1
+	−	+	5
+	−	−	15
−	−	−	6

+ = visible lesions.
− = no visible lesions.

TABLE 2

EXAMINATION OF 40 ISONIAZID-RESISTANT STRAINS ON VIRULENCE IN GUINEA PIGS

Survey of the Histologic Changes in Spleen and Liver of the Guinea Pigs, Eight Weeks After Injection

Organ	Macroscopic Changes	Histologic Changes (Pointing to Tuberculosis)	Ziehl-Neelsen Slides	Number
Spleen	+	+++	+	7
	+	+++	−	1
	+	++	++	1
	+	++	−	1
	+	+	−	4
	−	++	−	1
	−	+	−	4
	−	−		21
Liver	+	+++	++	1
	+	+++	+	7
	+	+++	−	1
	+	++	+	2
	+	++	−	1
	+	+	−	3
	+	−		3
	−	+++	+	1
	−	+++	−	2
	−	++	+	1
	−	++	−	5
	−	+	−	2
	−	−		11

Macroscopic changes: + = visible changes; − = no changes.

Histologic changes: +++ = very marked changes; ++ = marked changes; + = slight changes; − = no changes.

Ziehl-Neelsen slides: ++ = many acid-fast rods; + = few acid-fast rods; − = no acid-fast rods.

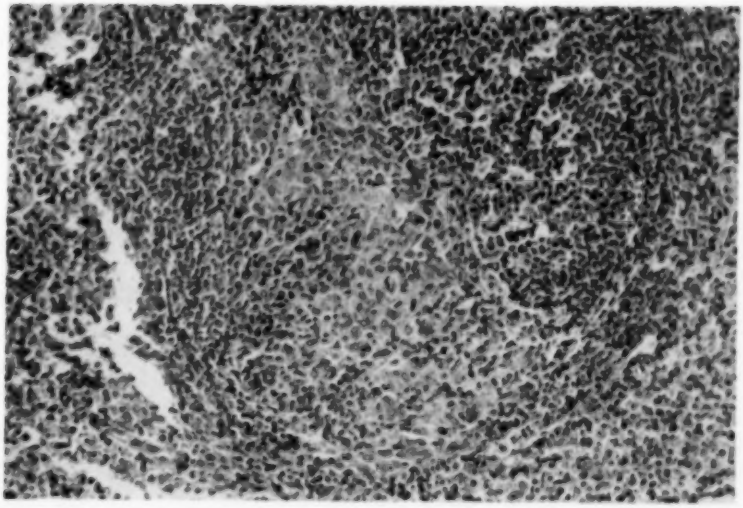

FIG. 1. Microphotogram of spleen of guinea pig showing infiltration of lymphocytes in healing tubercle. (Magnification 150 ×)

As may be seen, in the case of 14 guinea pigs, neither the macroscopic nor the microscopic examination revealed any abnormalities in the spleen or the liver; nor were these found in the lungs of the animals examined.

Just as in the tuberculosis caused by virulent tubercle bacilli in the guinea pig, the tuberculous changes in the spleen of the guinea pigs first—and in most cases exclusively—occurred in the follicles. The centers of these consisted of a more or less extensive accumulation of epithelioid cells with only a few lymphocytes. Sometimes these accumulations of epithelioid cells in the follicles were so large as to leave only a narrow zone of lymphoid tissue on the periphery of the tuberculous focus.

Unlike animals with normal tuberculosis, those infected with isoniazid-resistant bacilli were sometimes found to have some giant cells of the Langhans' type in the foci; one guinea pig showed considerable calcifications in the center of the epithelioid cell tubercle. In some guinea pigs the extent to which the epithelioid tissue had been infiltrated by lymphocytes, especially on the periphery of the focus, was striking. In other cases the tubercle had, as it were, been divided into smaller fields by zones of lymphocytes, and sometimes only a few scattered epithelioid cells could be found in an otherwise apparently normal follicle (figures 1 and 2).[7]

In the liver too, tubercles which were situated in the periportal regions consisted for the most part of epithelioid cells. The nuclei of the epithelioid cells had a dark color and were less vesicular than normal and at the same time the formation of connective tissue in and around the foci was sometimes so marked that the disease might be said to have been encapsulated (figure 3). Occasionally, all that was left was a small nodule of connective tissue with a few epithelioid cells.

Histologic examination of the lungs frequently revealed small foci consisting of a few

[7] The microphotos were made in the biophysical laboratory of the National Institute of Public Health by J. de Beus.

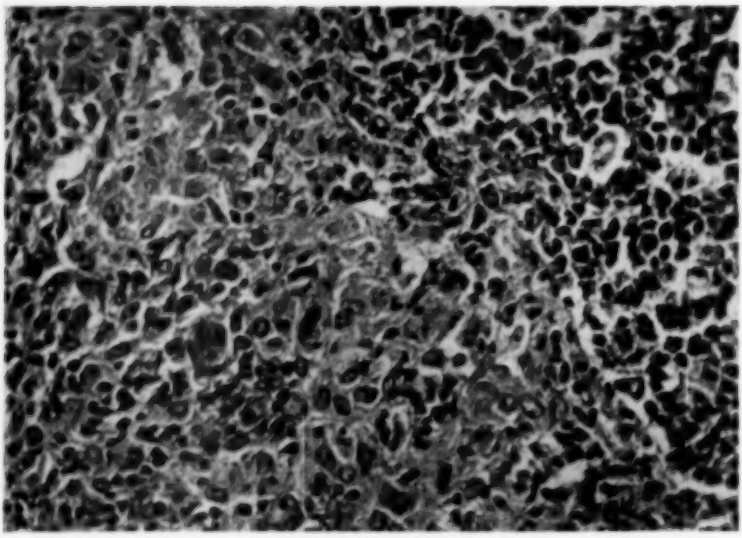

FIG. 2. Microphotogram (magnification 375 ×) of same spleen as in figure 1.

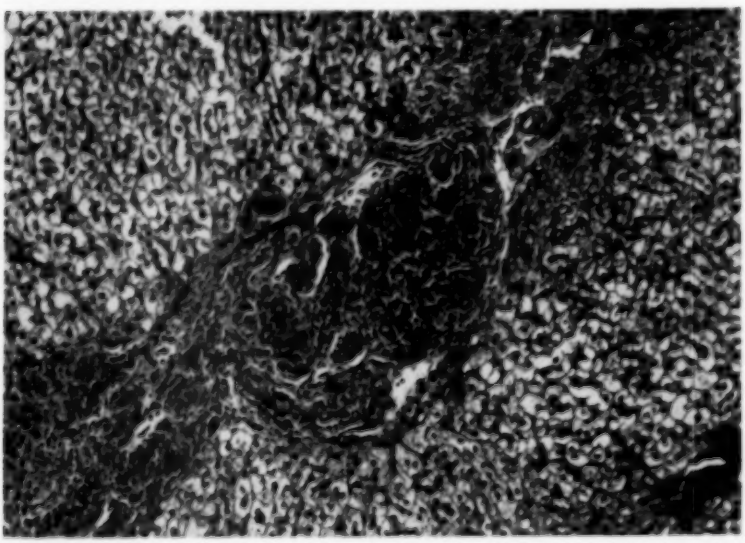

FIG. 3. Microphotogram of liver of guinea pig, showing encapsulation of healing tubercle. (Magnification 140 ×)

alveoli. In these foci the alveolar structure had been retained, the alveoli themselves being filled up with cells resembling epithelioid cells and sometimes with large polynuclear giant cells. As no acid-fast rods were even found in these foci and the cells sometimes contained a black pigment, there is no certainty that these foci were of a tuberculous nature.

The findings in the guinea pig after the injection of isoniazid-resistant tubercle bacilli differed not only clinically but also pathologically and anatomically from those of the common experimental tuberculosis and were an indication of the reduced virulence of these bacilli. This is proved by the fact that some isoniazid-susceptible and, therefore, virulent strains, isolated from patients before the treatment with isoniazid, did kill the guinea pig, whereas strains isolated from the same patients after this treatment did not. It is also proved by the increase in weight of 30 to 40 per cent observed in the course of the experiment in some guinea pigs whose organs were shown by the autopsy to have been morbidly changed. The macroscopic changes in the organs of the killed guinea pigs were less marked than in normal tuberculosis. In the liver, for instance, the typical infarct-like yellowish white foci were never found.

Histologically, in the changes in the organs of guinea pigs, experimentally infected with virulent tubercle bacilli, beautiful epithelioid cells with light-colored vesicular nuclei were found to predominate, with a few fibroblasts and lymphocytes. Giant cells of the Langhans' type rarely occurred and no calcification was seen.

Fundamentally, the histologic changes in the guinea pigs infected with isoniazid-resistant tubercle bacilli did not differ greatly from the normal picture of tuberculosis. Sometimes the epithelioid cells, especially those in the liver, had a darker and less vesicular nucleus; along the periphery of the foci there sometimes was a distinct encapsulation by connective tissue. In the spleen lymphocytes were occasionally found to have penetrated into the foci of epithelioid cells. Giant cells of the Langhans' type occurred fairly frequently, while in some spleens there was a calcification in the center of the foci.

This penetration of lymphocytes and the formation of a connective-tissue capsule should, it is believed, be regarded as a form of the recovery process.

In summary: In the guinea pigs infected with isoniazid-resistant strains, as in those infected with virulent tubercle bacilli, the regional and lumbosacral lymph nodes were nearly always found to have undergone a change (enlargement, caseation, softening).

Macroscopic lesions of spleen (enlargement, formation of tubercles) and liver (formation of tubercles) were found only in 19 of 40 animals. These lesions were far less extensive than those found in normal tuberculosis.

Unlike the progressive, sometimes centrally degenerated, epithelioid cell tubercles after an infection with virulent tubercle bacilli, the tuberculous lesions brought about by isoniazid-resistant strains were far less extensive and gave evidence of a considerable degree of healing.

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March 29, 1956

TAURINE IN THE TREATMENT OF TUBERCULOSIS IN GUINEA PIGS

Takeoka[1] in 1917 found that the amino acid taurine had an inhibitory effect on experimentally induced tuberculosis in guinea pigs. As no other work using this amino acid on experimental animals has been reported, the present study was undertaken to re-evaluate Takeoka's results. A comparison of the effectiveness of taurine and streptomycin was also made.

A group of 24 guinea pigs was used in this experiment. They were all of the male sex and each was approximately 400 gm. in weight. The animals were divided into the following four groups of 6 animals each: pretreatment controls, taurine recipients, streptomycin recipients, and final controls.

All guinea pigs were inoculated with a standardized suspension of H37Rv prepared in the following manner:

Two loops of a fifteen-day-old culture of H37Rv were removed from a slant of American Trudeau Society medium and placed in a sterile 15 ml. centrifuge tube. A sterile glass rod with rounded ends was used as the grinding instrument. Sterile phosphate buffer (Na_2HPO_4), was added gradually as a diluent while grinding. When the buffer had reached a volume of 5, the tube was allowed to stand for thirty minutes and the supernatant was transferred to another tube in order to get rid of clumps. From this new tube a 1 ml. aliquot was taken and spun in a Hopkin's vaccine centrifuge tube for one-half hour at high speed. The weight of bacilli present was calculated at 1 mg. per cu. mm. of packed cells. The contents of the 15 ml. graduated centrifuge tube were then increased by the addition of buffer, so that there was 0.1 mg. of bacilli per ml. This method is one previously described by Willis and Cummings.[2]

Each of the 24 guinea pigs was given a subcutaneous inoculation of 0.1 ml. of the suspension containing 0.01 mg. of the H37Rv organism.

The infection was allowed to progress for one month in all animals, and then, in order to check on the development of the disease, a group of 6 animals, the pretreatment controls, was sacrificed. These animals showed satisfactory establishment of the disease (figure 1). Treatment was started with taurine on one group of 6 animals while streptomycin was given to another group of 6 animals. In the remaining group of 6 animals, the final controls, the infection was allowed to continue its natural development. These animals served as the controls and represented the unaltered course of tuberculosis in guinea pigs.

The medications were given daily for a two-month period. The streptomycin group received 6 mg. of streptomycin dissolved in 1 ml. of sterile normal saline. The taurine group received 60 mg. daily of taurine dissolved in 1 ml. of water. This solution was boiled. All medication was injected subcutaneously into the left groin.

The weight of the animals in the different groups did not vary significantly. The incidence and extent of gross involvement of lungs, liver, spleen, and lymph node are shown in figure 1. The key for the symbols used in the figure is that of Feldman and Hinshaw[3] modified as explained in the legend. Microscopic examination of these same organs cor-

[1] Takeoka, M.: J. Infect. Dis., 1917, *20*, 442.

[2] Willis, H. S., and Cummings, M. M.: Diagnostic and Experimental Methods in Tuberculosis, Charles C Thomas, Springfield, Illinois, 1952, p. 171.

[3] Feldman, W. H., and Hinshaw, H. C.: Am. Rev. Tubere., 1945, *51*, 582.

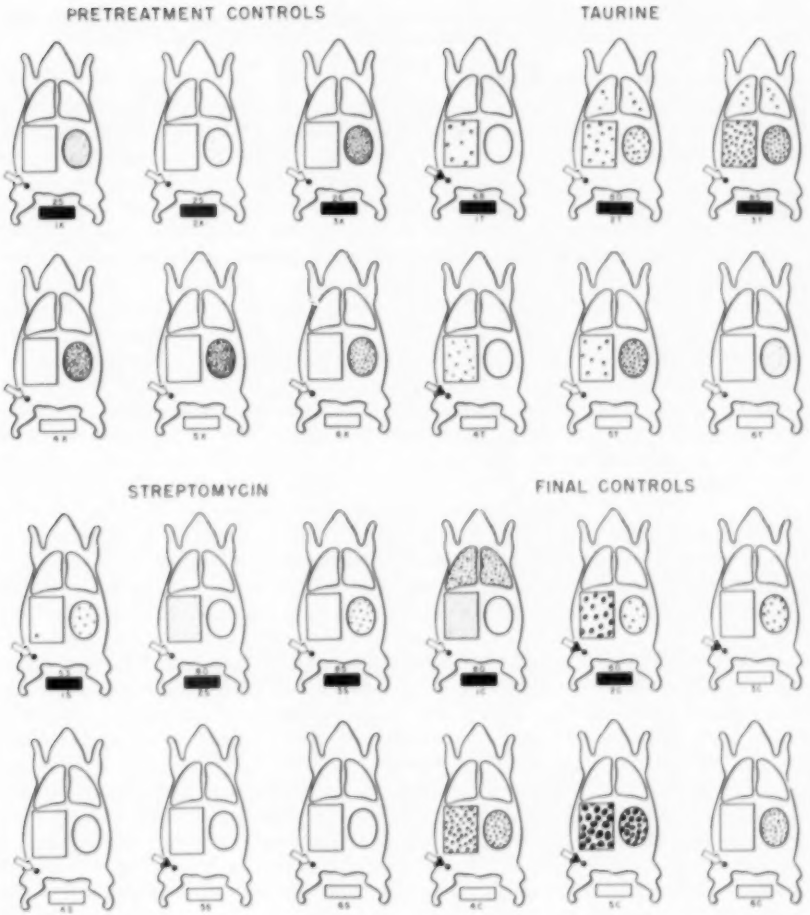

FIG. 1. The conventional symbols are used, with the exception that animals that lived for the full term of the experiment have a white rectangle drawn beneath them, while those which died before the term of the experiment have a black rectangle. The number above the rectangle shows the duration of the infection in days.

related well with the macroscopic appearance. Bacilli were recovered by culture equally from the taurine and control groups, but much more infrequently from the streptomycin group.

In summary: It was not possible to demonstrate any beneficial or inhibitory effect of taurine on the tuberculous process in a small group of guinea pigs. The previous report on the antituberculous activity of this compound is thus not confirmed. No information

concerning combination of taurine with other drugs, i.e., streptomycin or isoniazid, was obtained.

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X-RAY THERAPY IN THE MANAGEMENT OF TUBERCULOUS CERVICAL LYMPHADENITIS

In an analysis of the results of X-ray irradiation as the sole method of therapy applied to sixty-five patients with tuberculous lymphadenitis, it was found that the over-all healing rate in the group was more than 50 per cent.[1] The report was written during the time of the established usefulness of streptomycin, isoniazid, and PAS, but the actual therapy analyzed had been given during a period of time antedating the general use of these drugs. In this previous report, emphasis was made of the fact that the 50 per cent effectiveness was not presented as a brief for the use of X-ray therapy alone in the treatment of tuberculosis of the lymph nodes, but as a strong indication that, if significantly useful when used alone, X-ray irradiation would be appreciably more effective as an adjunct to antimicrobial or surgical therapy.

For years, there was a generally expressed fear of the use of X-ray irradiation because it was believed that acute dissemination of the disease often followed. Disseminated forms of tuberculosis were not observed in the above-mentioned series, or in other series referred to in the previous report. Certainly, with the antimicrobials presently available, dissemination should not be feared at all, except in those patients who are toxically ill, or in patients with already established disseminated tuberculosis, in whom the local therapy of cervical adenitis would likewise not be expected to be of value.

The purpose of the present report is to describe the management of tuberculosis of the cervical lymph nodes in 7 cases in which the disease had failed to respond satisfactorily to antimicrobial and/or surgical therapy. Because of the low prevalence of this form of tuberculosis, large series of cases are only rarely assembled, and hence it is believed that the results even in a small series are worthy of detailed study.

The patients were seen in one of the chest clinics of the Department of Public Health of the city of Philadelphia. All of the patients were Negroes. Only one patient had pulmonary tuberculosis along with the adenitis. He had been treated with pneumoperitoneum and antimicrobials for concomitant pulmonary disease, which was thought to be stable when he was referred to the clinic. In all of the patients the cervical lymph nodes were the site of involvement. In 3 patients, confirmation of the etiologic diagnosis was obtained by culturing tubercle bacilli from pus draining from the lesions. In the other 4 patients, the diagnosis was confirmed by histologic examination of biopsy specimens taken from the lesions. All of the patients had received some form of therapy previous to being referred to the clinic. Two patients had received previous antimicrobial therapy alone, 2 had had previous incision and drainage alone, while 3 had had surgery (2 had incision and drainage, one had extirpation) and antimicrobial therapy. At the time of referral all of the patients were in good general physical condition, without fever, signs of toxicity, or malnourishment.

The X-ray therapy given consisted of irradiation twice a week for a total of five exposures. Each irradiation delivered 100 r (in air) generated by 200 kilovolts constant potential, with 0.5 mm. copper added filtration and a half-value layer of copper.

The antimicrobial therapy which was either started or continued by the clinic was given over at least a six-month period. Further details concerning the 7 patients are best presented by short summaries of the individual cases:

B. J., a 16-year-old female, had been treated for pulmonary tuberculosis for six years prior to referral to the clinic. Treatment had consisted of left phrenic crush, pneumoper-

[1] Acreto, J. N., *et al.*: Am. Rev. Tuberc., 1953, *68*, 157.

itoneum and, later, courses of streptomycin-PAS and isoniazid-PAS. The pulmonary disease was considered stable when referred to this clinic. Three months before referral, while still receiving isoniazid-PAS, the patient noted swelling, gradually increasing fluctuation and, finally, draining of cervical lymph nodes.

Upon referral to the clinic, the isoniazid-PAS therapy was continued. She was given one course of X-ray irradiation. The swelling had completely subsided three weeks following this course. There was still a small amount of intermittent draining, however, and a second course of X-ray irradiation was given. Three months after the start of the first course of treatment, there was healthy scar tissue without swelling or draining. The same findings were noted at the six-month follow-up examination.

C. H., a 16-year-old male, had had swelling and draining on the right side of his neck three years prior to referral to the clinic. This condition was treated by X-ray irradiation at another institution. The swelling was greatly reduced in size, and the area was finally excised. The tuberculous etiology was confirmed by histologic study. The site remained healed.

One year before referral to the clinic, the patient began to have pain, followed by swelling on the opposite side of his neck. The diagnosis was established at the same institution by a positive culture for tubercle bacilli from the draining material. He was given three months of isoniazid-streptomycin, but without change in the status of the lesion. He was then referred to the clinic, and received one course of X-ray therapy while antimicrobial therapy was continued. Two weeks following the last irradiation, only several small "shotty" nodes were noted beneath two pin-point scars. Five weeks were required from the onset of X-ray therapy for these results. Six-month follow-up examination revealed no change from the immediate post-therapy findings.

E. R., a 36-year-old male, began to have swelling and draining on the left side of the neck three months before referral. The diagnosis was established by a positive culture for tubercle bacilli from the draining pus. Incision and drainage were performed, and antimicrobial therapy was advised. The patient did not comply, however. The swelling and draining persisted, and he returned to his District Clinic, and was then referred to this clinic. One course of X-ray therapy was given, and isoniazid-PAS therapy was started. Two weeks after the last X-ray dose was given (a total of five weeks of therapy) there were only two small "shotty" nodes remaining. After six months no change in this finding was noted.

W. J., a 19-year-old male, began to notice a swelling on the left side of his neck six months before referral here, following a tooth extraction from the left lower jaw. This swelling gradually progressed and finally began to drain. Incision and drainage were performed two months before referral to this clinic. When first seen here, a positive culture for tubercle bacilli had been obtained, and it was found that the patient was experiencing pain and limitation of movement of the jaw. The swelling was noted to be approximately 6 cm. in diameter. Isoniazid-PAS therapy was started, and a course of X-ray therapy was given. After two irradiations, the pain had completely subsided. By the time the patient had finished the fifth irradiation, the swelling had diminished to 2 cm. in diameter. Approximately two months from the onset of therapy, there was no swelling, with only a small scar to be seen. There was no change from this finding at the six-month follow-up visit.

L. G., a 14-year-old female, first noted swelling of the cervical lymph nodes on the left side of her neck four years before referral to this clinic. After two years of continued swelling, this area was excised, but swelling and draining recurred one year later. Once more, it was excised, and the patient was given daily streptomycin-PAS therapy for three months. The patient remained well until one month before referral to this clinic, when the area again began to swell and drain. Upon referral to the clinic, the antimicrobial therapy was continued, although later was changed to an intermittent dosage of streptomycin, and a course of X-ray therapy was given. Again, in approximately two months from the start of therapy no swelling was noted, and only a small, solid scar remained. Six months later, the same scar, of normal appearance, without swelling, was noted.

C. H., a 15-year-old female, first noted swelling on the left side of the neck five years before referral to the clinic. Four months previous to referral, there was a sudden increase in size with the onset of fluctuation. Incision and drainage with biopsy were done at another institution. The biopsy confirmed the tuberculous etiology. At the same time, isoniazid-streptomycin therapy was started. Swelling and draining continued, however, and the patient was referred to this clinic. Antimicrobial therapy was continued, and X-ray therapy was started. One week after the final irradiation, no swelling was apparent and the draining had stopped. This represented a period of three and one-half weeks from the onset of treatment. Six months later, only two small scars were seen.

F. N., a 15-year-old female, began to have a swelling on the right side of her neck nine months before being seen at this clinic. This rapidly became fluctuant and began to drain. She first went to another institution for treatment, and incision and drainage with biopsy were performed; examination of the specimens confirmed a tuberculous etiology. As no improvement ensued, streptomycin-PAS therapy was started, and continued for six months before referral of the patient to this clinic. On examination here, a fluctuant swelling, the size of a walnut, still profusely draining, was noted. Antimicrobial therapy had been discontinued by the patient for one month. Isoniazid-PAS therapy was started, and a course of X-ray therapy was given. Two months after the onset of treatment, only a pea-sized swelling was noted, and there was no draining. Antimicrobial therapy was continued for six months. At the end of that time no swelling was present; only a healed scar was noted.

Comment: It may be seen that all 7 patients have achieved excellent results from the addition of X-ray therapy to antimicrobial therapy. In 5 cases this form of treatment followed surgical therapy. It is noteworthy that these results were obtained within approximately three and one-half weeks in one case, five weeks in 2 cases, and two months in 3 cases. The longest period of time required from the onset of treatment to healing was three months (one case). No evidence of local side-reactions or spread of the disease was noted in any of the patients herein discussed.

To reiterate, it is agreed that tuberculous lymphadenitis is only one form of a systemic infection. Admittedly, the treatment of choice consists of combinations of antimicrobial agents used for long periods of time, together with good nutrition and proper hygienic conditions.[2] These are effective in treating both the generalized and local forms of the disease, although considerable time is often required in healing the local disease. If there is effective local therapy which will shorten the time required for healing, its use is decidedly indicated.

Surgical extirpation may be the best local therapy, particularly in cases marked by large, well-delineated swelling. In a recent paper Lester and Jones[3] once more reiterate this opinion and underscore the necessity for complete removal of the nodes. Nevertheless, there have been recurrences following both antimicrobial and/or surgical treatment. The above-mentioned writers suggest that X-ray therapy might be most useful in cases which had failed to respond to the above methods of therapy.

It should be added that, as described in the present report, the results of X-ray irradiation of involved nodes which had failed to respond favorably to antimicrobial therapy and/or surgery strongly indicate this method as an adjunct in the *local* therapy of this disease. Furthermore, it is believed that best results would be obtained using X-ray irradiation in combination with antimicrobials, and with surgery if necessary. Irradiation with X-rays appears to be a safe measure when used in a patient who is not debilitated or toxically ill from his tuberculosis.

In summary: This report gives the results of adjunct X-ray irradiation of diseased areas

[2] Muschenheim, C.: Am. Rev. Tuberc., 1954, *70*, 949.

[3] Lester, C. W., and Jones, J. M.: Am. Rev. Tuberc., 1956, *73*, 229.

in 7 patients with tuberculous adenitis who were considered as therapy failures when treated with antimicrobial agents and/or surgery.

The degree of healing in all 7 patients, the relatively short time required to achieve this, and the apparent safety of X-ray irradiation indicate this form of treatment as an adjunct to the two established methods.

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June 29, 1955

[4] The writer is grateful to Dr. M. J. Sokoloff, Chief, Department of Tuberculosis, City of Philadelphia Department of Public Health, for his aid in making this report possible; and to Dr. B. P. Widmann, Chief, Department of Radiology of Philadelphia General Hospital, and to his residents, for the X-ray therapy given to the patients reported herein.

Books

TUMEURS BRONCHO-PULMONAIRES. Exposés Anatomo-Cliniques. By A. Policard *et al.* Pp. 146, with illustrations, Masson et Cie., Paris, France, 1955. Price, 1,000 fr.

This little volume is commendable as a type of medical publication that is unusual in North America. It is a relatively inexpensive paper-backed edition, printed on glossy paper and illustrated with 92 black and white photographs. Unfortunately, however, the attractiveness of the package is not matched by the quality of the contents.

The work is essentially a series of papers, each dealing in a rather general way with some special aspect of primary pulmonary neoplastic disease. The articles are arranged in a more or less logical sequence, but there is little other evidence of the guiding hand of an editor in the assembling and presentation of material. Authors of some of the sections, for example, bury their references in the body of the text, whereas others list a bibliography at the end of the article. Some of the presentations refer to works whose source is not given. This defect seriously impairs the value of the book as a reference work. A further deficiency is the absence of a subject index. Lack of editorial control is further demonstrated by the illustrations. Although these are generally sharp and well printed, it is disturbing to find roentgenographic reproductions printed sometimes in positive, sometimes in negative, for no reason that is obvious.

The material presented in the different sections of the book does not give, and perhaps is not intended to give, complete and balanced coverage of the subject. There are, for example, sixteen pages on the principles, technique, and interpretation of diagnostic cytology, but only slightly more than a page on roentgenographic diagnosis. The latter section gives attention to such procedures as angiography and pneumomediastinum, but makes no reference to tomographic studies.

The section on the morbid anatomy of bronchopulmonary tumors is of value in its attempt to correlate the gross and microscopic characteristics of different types of neoplasm. It is pointed out, for instance, that massive tumors of homogeneous composition are almost invariably anaplastic carcinomas, whereas the majority of small tumors, with a diameter of less than 5 cm., are squamous carcinomas. Though these facts will be well known to most pathologists, such correlations should be of value to clinicians whose main acquaintance with pulmonary neoplasms is macroscopic.

In spite of the shortcomings noted above, the book should be of value to those dealing with pulmonary tumors so long as they do not demand of it complete encyclopedic coverage of the subject.

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BRONCHUS UND TUBERKULOSE. By A. Huzly and F. Böhm. Pp. 138 and 258 figures. Georg Thieme Verlag, Stuttgart, 1955.

Based on the experience of a large number of bronchoscopies and bronchographies of tuberculous and nontuberculous material, the authors systematically describe the bronchial changes in different stages of tuberculosis. Fifty-five case reports make the presentation instructive. As usual, in books published by Georg Thieme Verlag, the paper, print, and typography are excellent. The presentation of the colored pictures, drawings, and photoroentgenograms is of a high quality. The literature review is extensive.

Unfortunately, the role of the newer antituberculous drugs has not been taken into

consideration. But this volume will be of particular interest to students of tuberculosis, radiology, and laryngology.

Ole Wasz-Höckert
Helsinki, Finland

BOOKS RECEIVED

The Changing Character of Tuberculosis. By the Joint Tuberculosis Council. Pp. 28, Joint Tuberculosis Council, Devon, England, 1955. Price, 1/–.

Anales de la Catedra de Patologia y Clinica de la Tuberculosis. Universidad de Buenos Aires Facultad de Ciencias Medicas. Pp. 157, Universidad de Buenos Aires Facultad de Ciencias Medicas, Buenos Aires, 1954.

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Supplement 1 to Atlas of Exfoliative Cytology. By George N. Papanicolaou. Published for The Commonwealth Fund by Harvard University Press, Cambridge, Mass., 1956. Price, $4.00.

Tuberculosis in the Commonwealth. Transactions of the Fourth Commonwealth Health and Tuberculosis Conference. Pp. 507, National Association for the Prevention of Tuberculosis, London, 1955. Price, 30s.

The Lung as a Mirror of Systemic Disease. By Eli H. Rubin. Pp. 288, Charles C Thomas, Springfield, Ill., 1956. Price, $12.50.

Principles of Chest X-Ray Diagnosis. By George Simon. Pp. 174, Butterworth & Co., Ltd., London, 1956. Price, 50s.

A History of the Therapy of Tuberculosis and the Case of Frédéric Chopin. By Esmond R. Long. Pp. 65, University of Kansas Press, Lawrence, Kansas, 1956. Price, $2.00.

American Trudeau Society

Medical Section of the National Tuberculosis Association

The Award of the Trudeau Medal for 1955

The Trudeau Medal for 1955 was awarded to Dr. Max B. Lurie at the annual meeting of the National Tuberculosis Association, held in New York, New York, May 21–24, 1956. The presentation was made by Dr. William H. Feldman.

DR. FELDMAN: In 1925 the Board of Directors of the National Tuberculosis Association approved a suggestion of the Committee on Medical Research that there be awarded not oftener than once yearly a medal to one who had made meritorious contributions to the knowledge of the cause, prevention, or treatment of tuberculosis. This symbol of recognition was to be known as the Trudeau Medal. A year later this suggestion was accepted by resolution and adopted by the Executive Committee. At the 23rd annual meeting of the National Tuberculosis Association held in Washington, October, 1926, the award was first presented. The recipient had been president of the National Tuberculosis Association during the preceding year and was one of the true giants of American science—Dr. Theobald Smith. Thus was initiated a yearly custom which has become an important tradition.

Today we pause in our preoccupation with scientific sessions, committee meetings, and the many items that make a visit to New York exciting, to honor one of our peers by making him the thirtieth recipient of this enviable symbol of our respect and esteem.

The one who has been chosen unanimously to receive the Trudeau Award this year, and whose name I shall disclose in a few moments, is known to all of you, and his contributions to the knowledge of tuberculosis are highly regarded throughout the world.

Before proceeding further with the scientific accomplishments that merited this award I wish to digress briefly and give a short "clinical history" of our recipient. He was born in Lithuania, endowed with little else than a priceless scholarly heritage. He came to America at the age of fifteen. After high school at Townsend Harris Hall he entered the College of the City of New York. His ambition was to study philosophy, but he soon realized that this highly respected discipline offered little that would provide a means of livelihood in the immediate or near future. "In despair (and I use his words) I determined to study medicine." This decision was influenced by "the idea that the most one can do is to alleviate the pain of human life; medicine offers that opportunity."

He entered Cornell University Medical College and served in the Student Army Training Corps. During his medical training he became distressed at the thought of practicing medicine because of what he considered would be his incompetence. Always conscientious to a fault, he feared that his lack of knowledge would endanger the lives of those whom he was expected to treat. Finally his dilemma was resolved. Fate intervened and a highly sensitive, reluctant young physician was spared the agony and vicissitudes of practicing medicine, eventually to become a highly productive and brilliant investigator. In the words of our medalist, "It was my good fortune to diagnose my own tuberculosis in the fourth year of medical school." (He probably would have made a good physician.) He attempted to "take the cure" and at the same time carry on an internship, with the predictable result; he finally had to spend full time as a patient in a tuberculosis sanatorium. It was there that he became interested in what was to be his future career—research in tuberculosis. So much for the "clinical" aspects of this gifted man.

What are some of the attributes that have characterized him as a person and as a scientist? Above all, he has a great compassion, a real concern for the unfortunate, and he has an intense aversion to all that denies freedom and justice to man. While he can be

militantly indignant concerning social evils, he believes in, and practices, good will. He is a man of much humility and utterly without pretense. He places a high value on integrity, believing as he has expressed it, "Of what avail is the acclaim of others if you cannot countenance yourself." He has a sense of humor, enjoys an argument and, parenthetically I would say, is a skillful debater. He considers differences of opinion healthy and desirable. His extra-scientific interests are social justice, philosophy, and music. The motivation of his labors has been to seek a meaning and a justification for life. In his quest he has succeeded admirably, to the envy of us all.

He has brought to his professional tasks a thoughtful, scholarly insight, an ingenious talent for experimentation, a masterly technique, and a fiery enthusiasm. Importantly, he has a fine concept of scientific evidence. He has stated the underlying philosophy of his research in the following words:

> I have always thought that it is better to attempt the solution of a fundamental problem and fail than to undertake the elaboration of minor details with great success.

These words are worth remembering.

The scientific contributions of the recipient of the Trudeau Medal for 1955 are sufficiently well known to require no enumeration. Briefly, he has been the sole or senior author of nearly 70 publications, including several of monographic dimension, and his name appears in joint authorship of many more. His scientific writing, especially his reviews, reveal a capacity to establish continuity of new facts with those previously reported. This bringing together of the new with the old enables the reader to obtain a composite understanding of the interrelationships of the various facets of the subject discussed.

Although the main theme of his many investigations has been resistance to tuberculosis, of outstanding importance is his brilliant conception and successful demonstration of the relationship of generic factors and resistance. One who knows him well has said, "His early genetic work opened up a new world." He has continued to explore this world successfully and with results that have brought him international renown.

The recipient of the Trudeau Medal for 1955 has, in a word, accomplished work which has been magnificent in its purpose, has, I am certain, been deeply satisfying in its accomplishment, and has brought to a devoted and dedicated servant of science a touch of immortality.

On behalf of the Trudeau Award Medal Committee, I consider it an honor and a happy privilege to present the Trudeau Medal Award for 1955 to Max B. Lurie.

Dr. Lurie: Mr. President, the Committee on the Trudeau Award, Dr. Feldman, ladies and gentlemen.

To merely say that I am profoundly moved by the recognition that you have given to our labors over the years would be an understatement. Indeed, I see in it a vindication of my hope that my life has not been altogether in vain. Even more, I take it as evidence of the great generosity of spirit that characterizes our democracy. For a poor immigrant boy to have received the necessary training, to be given the opportunity to pursue his craving to try to understand even an iota of the mystery of life is a triumph of the human spirit. Most of all, for the Trudeau Society to award this distinguished honor to one who has achieved no immediately practical solution to the problems on hand, but to him who has endeavoured by tenacity and devotion to penetrate ever so little into the intricacies of the host-parasite relationships which constitute native and acquired resistance to tuberculosis,

is both a humbling thought and a warming of the heart. It is humbling, for we all know the ephemeral nature of even the loftiest and most penetrating of scientific ideas. The centuries' old sway of Newton's laws over the universe has yielded to the relativistic concepts of Einstein. It is a sacrilege to mention these giants of the human mind in the same breath with our feeble efforts. But the condition of human existence is the same in the great and in the small; absolute knowledge is beyond our capacity. It is in increasing understanding that the salvation of man lies. Not only in his mastery of his environment, in the subjugation of the elements to his will, as we are witnessing daily in ever increasing crescendo, but even more in the control over himself and the moral growth of mankind. The more man understands himself the more truly human will he become. It is heartening that the Trudeau Society deems it worthy to encourage these labors, assailed as they often are by failure and doubt.

I wish to take this opportunity to express my gratitude to the Henry Phipps Institute of the University of Pennsylvania, to my teachers and inspirers within and outside its walls, to my wife who has stood unflinchingly by me in times of trial, to the organizations that have supported these endeavours over the years, especially to the Commonwealth Fund, the Trudeau Society, and the Public Health Service, and above all to my collaborators and assistants, without whose devotion even the little we did could not have been accomplished.

On behalf of all those whose greatest joy in life is the glimmer of understanding that comes after searching and critical analysis, allow me to thank you for your generosity.

Obituaries

DANIEL W. ZAHN

1911–1956

Daniel W. Zahn, Medical Director of Firland Sanatorium, Seattle, Washington, died on May 6, 1956, of cerebral thrombosis complicating a recent thrombosis of a coronary artery. His inspirational leadership will live long in the hearts and minds of his colleagues, friends, and family. The field of tuberculosis lost a dedicated physician, scholar, and leader.

Dan Zahn was born May 2, 1911, in Brooklyn, New York, and attended college and medical school at the University of Glasgow, Scotland, receiving a B. S. in 1934 and an M. D. in 1938. Thereafter he returned to the United States, interned at Bellevue Hospital and remained as Assistant Resident in Medicine and, subsequently, Resident in Chest Diseases under J. Burns Amberson. A period of private practice was interrupted by military service at Fitzsimons General Hospital. Thereafter he served as Chief of the Tuberculosis Section at Fort Logan, Colorado, and joined the Firland Sanatorium staff in 1948. Here there followed a rapid ascendancy to Chief of Service and, in November, 1954, Dr. Zahn was appointed Medical Director, in which capacity he was serving at the time of his death.

Deeply interested in teaching, Dr. Zahn was Clinical Associate Professor of Medicine at the University of Washington School of Medicine and devoted boundless energies to instruction in chest diseases; students thrived on his general proddings. He authored many papers on tuberculosis and diseases of the chest. Active in numerous professional organizations, he was a Diplomate of the American Board of Internal Medicine and a Fellow of the American College of Physicians. In the recent year he served as President of the Western Tuberculosis Conference, President of the Pacific Northwest Trudeau Society, and President of Chest Survey, Incorporated. His professional activities were numerous, relating his scholarly approach to medicine to his insatiable appetite for knowledge. A deep sense of obligation to his fellow physicians led him to give tirelessly to the teaching of chest disease, and his dedication to mankind spurred his fight on tuberculosis.

Dr. Zahn is survived by his wife Charlotte, and two children, Marcia and Geoffery. With his passing goes a dedicated physician—scholarly, searching, serving, who truly practiced the art and science of medicine. His memory will long be cherished by those who served with him and the patients whom he served. He will long furnish inspiration to those with whom he worked.

T. F. S., Jr.

WILLIAM WARNER WATKINS

1883–1955

Arizona has lost another of its pioneer "tuberculosis fighters," and one of its most prominent and helpful physicians in the field of chest diseases.

William Warner Watkins died in Phoenix, Arizona, at the age of seventy-two; he had been a resident of the state for exactly fifty years.

Dr. Watkins was born in Keysville, Virginia, in 1883. He was graduated from the Medical College of Virginia in 1906 and moved to the no longer existent town of Metcalf Arizona, in the same year. In 1907 he moved to Phoenix, where he had been an active physician and citizen ever since.

He was instrumental in the formation of the Arizona Association for the Study and Prevention of Tuberculosis on May 22, 1909, a society which later became the Arizona Tuberculosis and Health Association. He remained active in the organization until his retirement from the directorate in 1954, and was an emeritus president. He was Arizona's representative director to the National Tuberculosis Association from 1945 to 1949 and helped to organize the Arizona Trudeau Society in 1946. The Arizona Tuberculosis and Health Association honored him in 1951.

Dr. Watkins started the first pathology laboratory in Arizona and was also founder of one of the first X-ray laboratories. He was active in Baptist Church work, the YMCA, service club work, and many other civic groups. He leaves a wife, a daughter, and three grandchildren.

W. H. O., Jr.

NOTICES

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of papers for the 1956 annual meeting to be held in Kansas City, Missouri, May 6–8, 1957.

Abstracts

1. Abstracts of papers to be offered for presentation should be submitted before January 8, 1957 to the Chairman of the Medical Sessions Committee. *SIX COPIES* are required for distribution to committee members.

2. Abstracts should be limited to a 300-word summary including important data *AND CONCLUSIONS*. Charts and tables should be attached to aid in the selection of papers.

3. The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper must be the first author listed in the abstract.

4. All abstracts will be reviewed by the Medical Sessions Committee. Final selection will be on a competitive basis.

5. Subjects for presentation may include all scientific aspects of tuberculosis and non-tuberculous respiratory and cardiopulmonary diseases.

6. Those wishing to present subjects of a confidential nature from the armed forces or other sources must first clear the material through official channels.

7. Essayists submitting acceptable surgical papers may present their papers at a surgical session Wednesday afternoon, May 8, 1957, if so desired, in order to avoid conflict with the meetings of the American Association for Thoracic Surgery.

Papers

1. Authors will be notified by March 1, 1957, if their papers have been accepted for presentation.

2. Papers presented at the Annual Meeting must be original contributions not previously presented or published. Speakers at the Annual Meeting who wish their paper considered for publication in THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASE may submit a double-spaced, typewritten manuscript *IN DUPLICATE* to the moderator of the session.

3. The time allotted for presentation of papers at the Annual Meeting will be strictly limited by the committee in order to permit adequate discussion.

4. Participants in the program are reminded of the fact that travel expenses to the Annual Meeting are not paid by the National Tuberculosis Association.

EDWARD J. WELCH, M.D., *Chairman*
Medical Sessions Committee
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NATIONAL LIBRARY OF MEDICINE

President Eisenhower on Friday, August 3, signed a bill establishing a National Library of Medicine in the Public Health Service and transferring to the Service all of the functions of the Armed Forces Medical Library.

In carrying out the provisions of the National Library of Medicine Act, the Surgeon General will be guided by the advice and recommendations of a Board of Regents. The Board will consist of seven ex officio members: the Surgeons General of the Public Health Service, the Army, Navy and Air Force, the Chief Medical Director of the Department of Medicine and Surgery of the Veterans Administration, the Assistant Director for Biological and Medical Sciences of the National Science Foundation, and the Librarian of Congress, and ten additional members appointed by the President with the consent of the Senate. The appointive members will serve four years.

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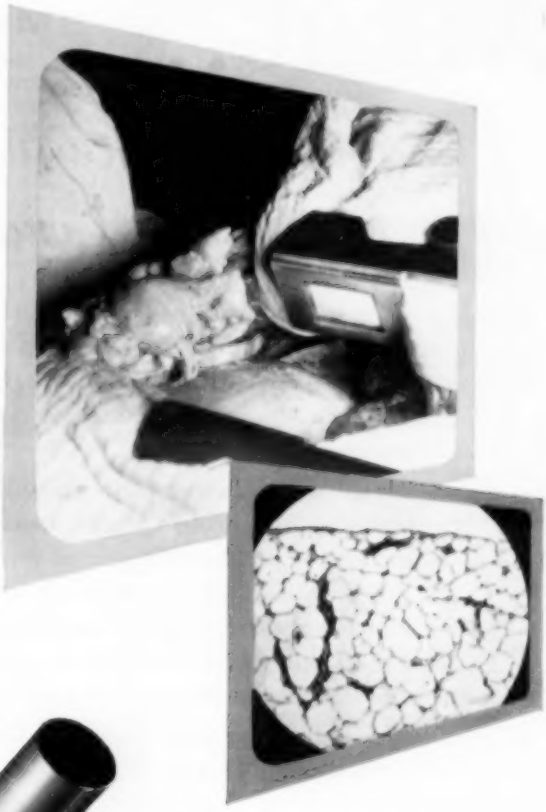

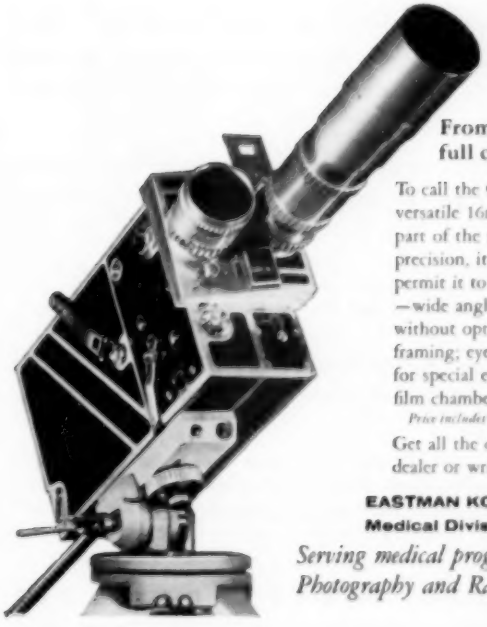

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1. Odel, H. M.: Nutrition in Cardiovascular Disease, in Wohl, M. G., and Goodhart, R. S.: Modern Nutrition in Health and Disease, Dietotherapy, Philadelphia, Lea & Febiger, 1955, p. 709.
2. Berg, C. P.: Utilization of Protein, J. Agr. & Food Chem. *3*:575 (July) 1955.
3. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, ed. 6, Baltimore, Williams & Wilkins, 1955, p. 638.

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*Miller, J.B., et al.: Ann. Allergy, 12:611, Sept.-Oct., 1954.

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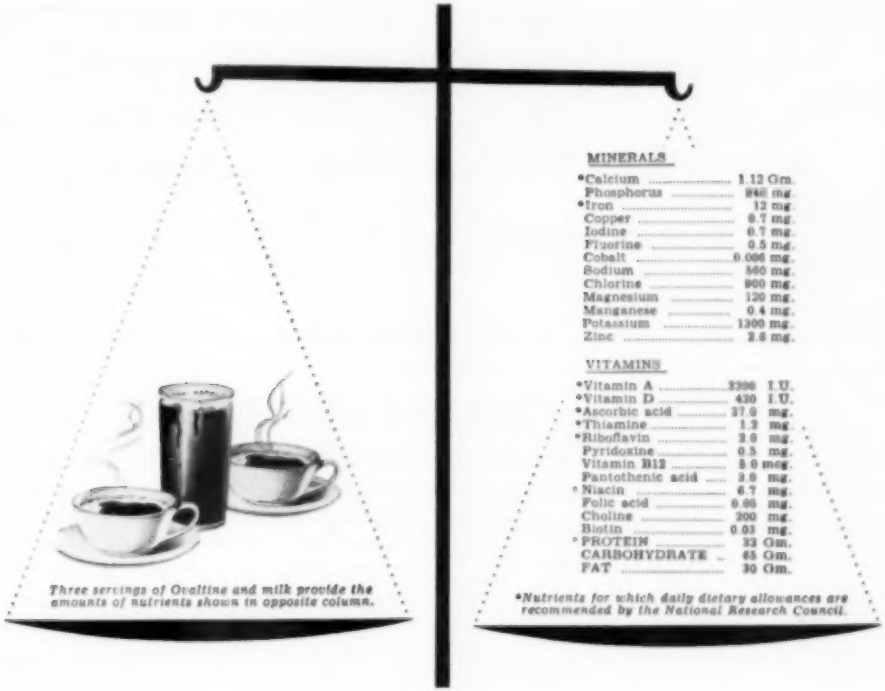

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Notes:
Some Observations on the Pathology of the Lesions Caused by Isoniazid-Resistant Tubercle Bacilli in the Guinea Pig. H. H. VINK *et al.* ... 633
Taurine in the Treatment of Tuberculosis in Guinea Pigs. NOBLE M. WRIGHT *et al.* ... 638
X-Ray Therapy in the Management of Tuberculous Cervical Lymphadenitis. JOSEPH N. ACETO ... 641
Books ... 645
American Trudeau Society:
The Award of the Trudeau Medal for 1955 ... 647
Obituaries ... 650
Notices ... 652
Abstracts ... 131

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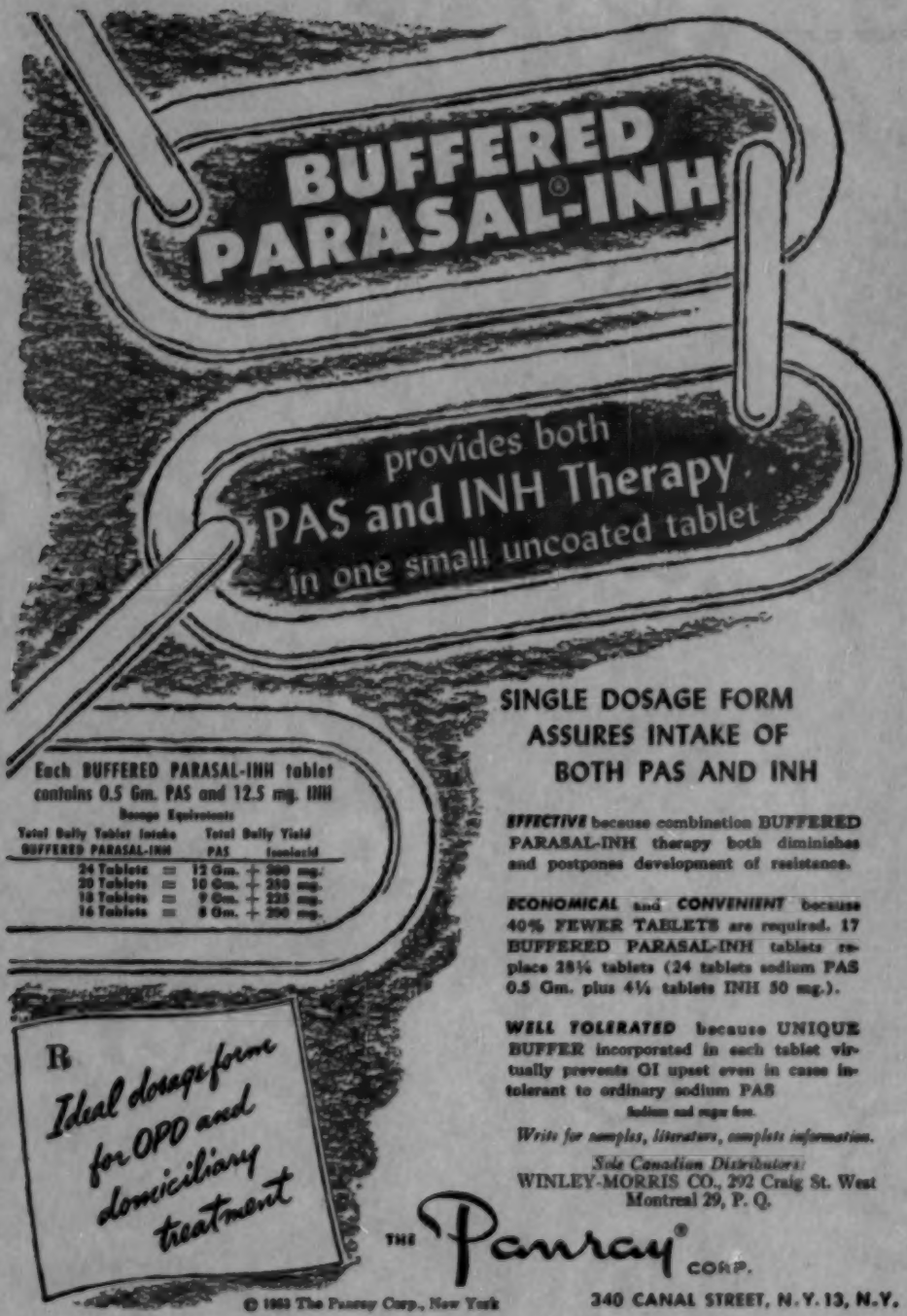
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